THE BIOLOGY OF *RHYACIONIA SUBTROPICA* MILLER (LEPIDOPTERA:OLETHREUTIDAE)

By

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THE BIOLOGY OF *RHYACIONIA SUBTROPICA* MILLER (LEPIDOPTERA:OLETHREUTIDAE)

by

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Chairman: Dr. R. C. Wilkinson
Major Department: Entomology and Nematology

The biology of *Rhyacionia subtropica* Miller, a tip moth which attacks young pines in Florida, was investigated.

R. subtropica was collected or recorded as attacking *Pinus elliottii* Engelm. variety *elliottii*, *P. elliottii* variety *densa* Little and Dorman, *P. palustris* M., and *P. thunbergiana* Franco in Florida; *P. elliottii elliottii* and *P. palustris* in southern Georgia; and *P. caribaea* Morelet at Freeport, Grand Bahama Island.

R. subtropica has 5 larval instars and the larvae are described and illustrated. The 4th and 5th female larval instars have paired secondary sex structures on their 8th and 9th abdominal sternites.

R. subtropica was bivoltine with a partial 3rd emergence in September and October, and the seasonal histories of both *R. subtropica* and *P. elliottii elliottii* exhibited intersynchronization.

The host tissues which larvae eat are described and how the various types of larval feeding affected *P. elliottii elliottii* growth are discussed.

Larval feeding sites were recorded more frequently in the upper portion of a flush and most frequently on the first two flushes of apical shoots. The percentage of infested pines within a height class increased as total height of 2-year-old pines increased.

The activities of 5th stage larvae prior to pupation are described. Pupation generally proceeded downward in multiple-infested shoots with an apparently random distribution of pupal sexes. The weight by sex, sex ratio, colors, and stadium duration of pupae are also reported.

The activities of encased pharate adults prior to eclosion, teneral adults during eclosion, and fully dried adults after eclosion are reported. The daily pattern of emergence under natural light cycle was bimodal, with major and minor peaks at 0858 and 1935 hours EDST, respectively.

Adults did not mate under laboratory or semi-artificial conditions, but field-collected larvae fed and developed on an artificial diet and produced pupae which issued adults.

Six species of hymenopterous parasites, *Bracon gemmaecola* (Cush.), *Temelucha* new species, *Hyssopus rhyacioniae* Gah., *Arachnophaga ferruginea* Gah., *Haltichella rhyacioniae* Gah., and *Sphilochalcis flavopicta* (Cress.), were recovered from individual *R. subtropica* larvae and pupae.

In fertilizer-insecticide experimental plots of 2-year-old bedded *P. elliottii elliottii*, the 0-P-K and 0-0-0 treatments, either with or without the phorate insecticide, were superior to any of the N-0-0 or N-P-K treatments for the avoidance of infestation by *R. subtropica*. Plots which received phorate had a significantly lower percentage of *R. subtropica*-infested pines.

Field and laboratory studies were conducted to also investigate the association of *R. subtropica* and pitch canker, a disease of pines caused by the fungus *Fusarium lateritium* f. *pini* (Nees.) Hepting. *R. subtropica* was concluded not to be a prime agent in the initiation and spread of pitch canker in *P. elliottii elliottii* in Florida.

A common name, subtropical pine tip moth, for *R. subtropica* was submitted to the Entomological Society of America.

CHAPTER I

INTRODUCTION

Shoot moth or tip moth larvae of the genus *Rhyacionia* mine and kill buds and shoots of pines. Until 1960, when *R. subtropica* Miller was described, *R. frustrana* (Comstock) and *R. rigidana* (Fernald) were the principal tip moth species known to attack pines in the southeastern United States.

In 1963, Merkel reported the status of knowledge about *R. subtropica* as follows: "Virtually nothing is known about its life history and habits, and it could be hazardous to assume that the biology of this species is the same as other tip moths, . . ." To date, the status of knowledge of *R. subtropica* includes morphological description of the adult (Miller 1960), identification characteristics of the pupa (Yates 1967c) and larva (Miller and Wilson 1964), host plants (Miller 1960, Miller and Wilson 1964), and geographic distribution (Miller 1960,1965; Bethune 1963; Frost 1963,1964; Miller and Wilson 1964; Kimball 1965); but the biology of *R. subtropica* remains unreported.

In recent years, five factors have aroused the need to investigate the biology of *R. subtropica*: (1) use of genetically superior seedlings to stock large acreage pine plantations, (2) application of fertilizer to existing and newly

established pine plantations (Pritchett and Smith 1970), (3) awareness that tip moths are the major factor killing conelets in some pine seed orchards (Yates and Ebel 1972, Ebel and Yates 1974), incrimination of tip moths in the disease cycle of pitch canker, a disease of pines caused by the fungus *Fusarium lateritium* f. *pini* (Nees). Hepting (Mathews 1962, Berry and Hepting 1959,1969), and (5) outbreaks of pitch canker in slash pine plantations and a slash pine seed orchard in Florida (Laird and Chellman 1972, Schmidt and Underhill 1974).

In 1970 University of Florida forest entomologists and pathologists, with technical assistance from private industry and the U.S. Forest Service, initiated studies to investigate the biology of *R. subtropica* and the relationship of *R. subtropica* and pitch canker disease in Florida.

The objectives of the *R. subtropica* investigation were to determine (1) geographic distribution in Florida, (2) number of larval instars, (3) seasonal history in Florida, (4) which host tissues larval instars eat and how feeding affects *Pinus elliottii* Engelm. variety *elliottii* growth, (5) pupation and pupal behavior, (6) emergence activities and daily emergence pattern(s), (7) mating, oviposition, and rearing media, (8) parasites, (9) the affect of fertilizer and insecticide on the incidence of infestation in *P. elliottii elliottii*,(10) distribution of larval feeding sites within the multinodal annual vegetative long shoot, and (11) incidence of infestation by tree height class.

To examine the association of *R. subtropica* and pitch canker, studies were conducted to determine (1) if *R. subtropica* is associated with pitch canker symptoms, (2) distribution of *R. subtropica* larval feeding sites and pitch canker symptoms on the multinodal annual vegetative long shoots of *P. elliottii elliottii*, (3) the effect of fertilizer and insecticide on the incidence of pitch canker in *P. elliottii elliottii*, (4) the association of pitch canker-producing *Fusarium* spp. and *R. subtropica* on pairs of *P. elliottii elliottii* shoots, and (5) which *R. subtropica* instar(s) is associated with the pitch canker-producing fungus. The results of these investigations are reported herein.

CHAPTER II

TAXONOMIC STATUS OF *RHYACIONIA SUBTROPICA*

Background

R. subtropica was first discussed as a new species by Miller and Neiswander (1959) and was described by Miller in 1960 from specimens collected in 1927. Miller (1960) also recommended the common name "Subtropical Pine Tip Moth" for *R. subtropica*. A common name has never been approved by the Committee on Common Names of Insects of the Entomological Society of America.

Merkel (1963) reviewed tip moth identification problems resulting from the belated recognition and description of this species. Miller (1960), Bethune and Hepting (1963), Frost (1963,1964), and Kimball (1965) reported pre-1960 studies which could have benefited from an earlier recognition of *R. subtropica*.

Yates (1967c) differentiated *R. subtropica* pupae from those of *R. rigidana* Fernald and *R. frustrana* (Comst.).

MacKay (1959) used previously undetermined specimens from the type-locality of *R. subtropica*, collected within ca. one month of the *R. subtropica* types, for the basis of her description of the larvae of *R. rigidana* Fernald [should be (Fernald)]. C. H. (MacKay 1959, Miller 1960) was Carl

Heinrich, and Heinrich's initials were on the labels of the specimens which MacKay (1959) used for the larval description of *R. rigidana* and which Miller (1960) designated as types for *R. subtropica*. Apparently, Heinrich recognized that both the larvae MacKay (1959) eventually used and the adults Miller (1960) designated as types were not specimens of *R. frustrana*, *R. rigidana*, or *R. buoliana* (Schiffermüller) because neither MacKey (1959) nor Miller (1960) reported species determination information when they (MacKay 1959, Miller 1960) cited label data. MacKay's (1959) description of *R. rigidana* larvae fits the larvae I collected in Glades County, Florida, that came from the same pine shoot as *R. subtropica* pupae which produced males and females whose genitalia and wing characteristics were identical to those characteristics described for *R. subtropica* (Miller 1960).

To prevent future taxonomic and nomenclatural complications, the approval of Miller's (1960) recommended common name has been initiated and the larva of *R. subtropica* is described.

Methods

Common Name Approval

After conferring with W. E. Miller, R. C. Wilkinson, and H. O. Yates III, I submitted, on 12 June 1974, the common name Subtropical Pine Tip Moth to G. D. Hertel, Chairman, Common Names Committee of the Southern Forest Insect Work Conference, for approval and recommendation to the Committee on Common Names of Insects of the Entomological Society of America.

Larval Description

Decapitated, 5th instar *R. subtropica* larval integuments were split longitudinally along the ventral midline, debrised, mounted in Hoyer's solution on microslides under 20 mm square coverslips, cleared, and illustrated with the aid of a vertically mounted microslide projector. Other larval instars were examined with a compound microscope and a dissecting microscope (magnification 40-100x).

The illustrations and description of the *R. subtropica* larvae were prepared following MacKay's (1959) format and chaetotaxy system to provide consistency of *Rhyacionia* larval information. The setal designations were omitted from the *R. subtropica* larval illustrations, but can be determined by referring to Figure 1 on page 168 in MacKay (1959). The dissected larval integuments prepared for and the stylized body segments used to illustrate the *R. subtropica* larva reduced the annulation of the body and expanded the longitudinal axis of the body, thereby distorting the body length of the larval illustration relative to the actual body length of a 5th stage *R. subtropica* larva. A scale (1 mm) accompanies the *R. subtropica* larval illustration.

R. subtropica larvae collected from Glades County, Florida, were deposited in the Florida State Collection of Arthropods, Gainesville. *R. subtropica* adults which emerged from pupae collected in slash pine shoots at Glades County, Florida, are located at the Department of Entomology, University of Georgia, Athens (C. W. Berisford, personal communication).

To the best of my knowledge, I have never seen a *R. rigidana* larva.

I have sent *R. subtropica* larvae and pupae to W. E. Miller and requested that he examine and compare the larvae of *R. subtropica* and *R. rigidana* during his current revision of the genus *Rhyacionia*.

Results

Common Name Approval

Subtropical Pine Tip Moth was approved by the 19th Southern Forest Insect Work Conference and will be recommended to the Committee on Common Names of Insects of the Entomological Society of America as the common name for *R. subtropica* (G. D. Hertel, personal communication).

Larval Description

The body length of *R. subtropica* larvae ranges from ca. 1.5 mm for the 1st instar to ca. 7 mm for the 5th instar. The range of head widths is 0.241-1.270 mm (1635 larvae) and the mean head widths (mm) for the 5 larval instars are 0.260, 0.345, 0.489, 0.721, and 1.044 (Figure 3 and Table 1).

The 1st larval instar head is brown with a darker lateral bar at the postgenal juncture; the body is pink to pale lavender; and the prothoracic setae L_1-L_3 are aligned linearly. The 5th larval instar head is yellowish-tan to golden with a dark brown or black lateral bar at the postgenal juncture; the body is ivory or cream colored; and the prothoracic setae L_1-L_3 are aligned linearly. The larval head becomes dark

brown prior to moulting and the body light pink following moulting in all larval instars. The prothoracic and anal shields' color are the same as and change along with the head's color.

The 5th larval instar abdominal segments 1-6 have 3 SV setae and segments 7-9 have 2 SV setae. Setae L_3 is absent on abdominal segment 9. Thoracic segments 2 and 3 have 4 extra setae; abdominal segments 1-8 have 3 extra setae, and abdominal segment 9 has 2 extra setae (Figure 1 C and D).

The 4th and 5th female larval instars have paired secondary sex structures on the venter of the 8th and 9th abdominal segments laterad of each V_1 seta and between the V_1 and SV setae (Figure 1 D, Figure 4, and Figure 5 B).

The head shape, head setal and ocellar patterns, body setae lengths, pinacula shape, extra setae and their placement, spiracular shape and alignment, and the integumental spinulation can be determined from Figures 1, 4, and 5. The spinneret, labial palps, tarsus, tarsal claw, and the tarsal setae are similar to those characters of *R. buoliana* (MacKay 1959).

Miller and Wilson (1964) discuss the characters for differentiating the larvae of both *R. subtropica* and *R. rigidana* from the larvae of *R. frustrana*. No characters are known for the separation of *R. subtropica* and *R. rigidana* larvae (Miller and Wilson 1964); and since I did not study the larvae of *R. rigidana*, the majority of the following studies were conducted in Glades County, Florida, outside the zone of

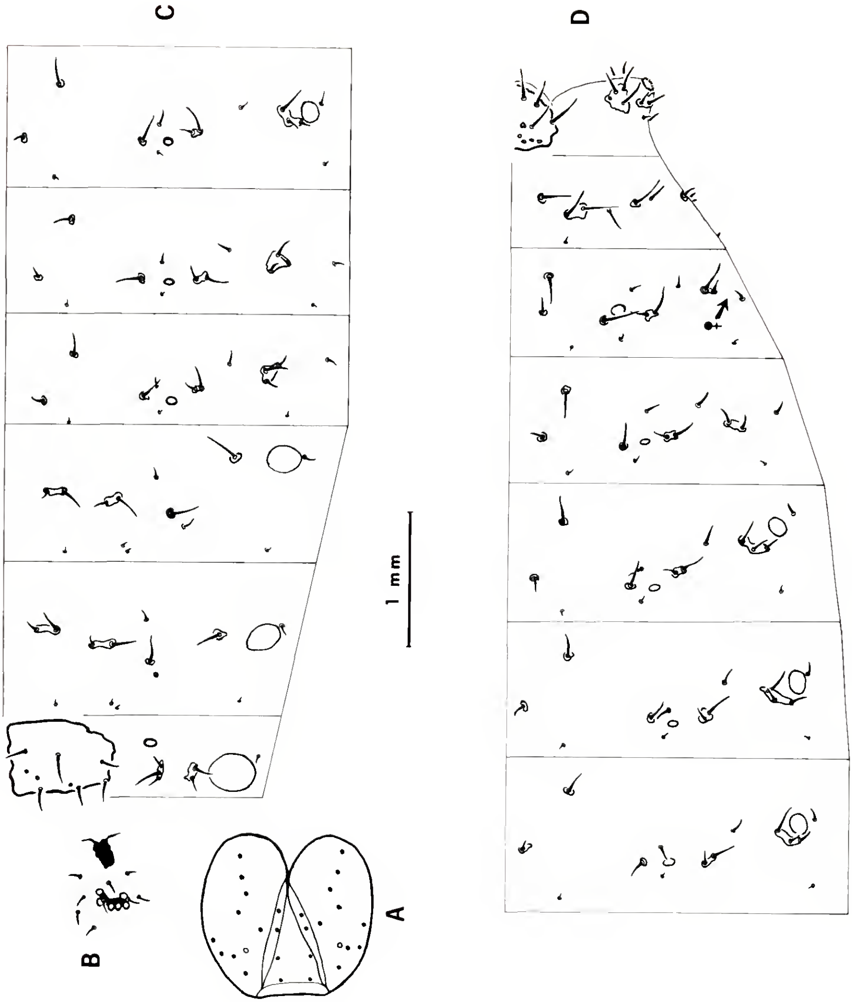
Figure 1. The 5th stage *R. subtropica* larva.

A. Dorsal view of head.

B. Lateral view of ocellar area of head with ocellar and setal patterns and with the lateral bar at the postgenal juncture.

C. Lateral view of the thoracic segments and abdominal segments 1-3.

D. Lateral view of abdominal segments 4-10, with an arrow on the 8th segment denoting the location of the secondary sex structure of female larvae.


C
D
1 mm
B
A

geographic distributional overlap of *R. subtropica* and *R. rigidana* to avoid problems of larval identification.

Discussion

I believe the above description of the *R. subtropica* larva, the morphology of the secondary sex structures on the female 5th larval instars, and W. E. Miller's current revision of the genus *Rhyacionia* will finally resolve the inability to separate *R. subtropica* and *R. rigidana* larvae. Also, I believe MacKay (1959) was probably examining *R. subtropica* larvae when she described the larvae of *R. rigidana*; therefore, I foresee *R. rigidana* (Fernald) (sensu MacKay 1959) becoming a synonym of *R. subtropica* Miller (1960).

Summary

R. subtropica was described by Miller (1960) and the name remains valid.

A common name, Subtropical Pine Tip Moth, for *R. subtropica* has been submitted to the Committee on Common Names of Insects of the Entomological Society of America for approval. The larva of *R. subtropica* are described and illustrated.

CHAPTER III

GEOGRAPHIC DISTRIBUTION AND HOSTS OF *RHYACIONIA SUBTROPICA*

Background

In the United States, the distribution of *R. subtropica* is reported to coincide with the distribution of slash pine varieties (Miller and Neiswander 1959, Miller 1960; Yates and Beal 1971, Baker 1972). Critchfield and Little (1966) and Little (1971) mapped the ranges of *Pinus* species.

R. subtropica is reported from Alachua, Baker, Bradford, Broward, Charlotte, Collier, Dade, Escambia, Flagler-St. Johns, Highlands, Hillsborough, Lee, Levy-Gilchrist, Manatee, Marion, Martin, Okaloosa (type-locality), Orange, Osceola, Palm Beach, Pinellas, Polk, Putnam, St. Lucie, Sarasota, Taylor, Union, and Volusia Counties, Florida; Lanier and Ware Counties, Georgia; Aiken County, South Carolina; and the southwestern counties of the Mississippi "boot-heel" attacking *Pinus elliottii* Engelm. variety *elliottii* (typical slash pine), *P. elliottii* variety *densa* Little and Dorman (south Florida slash pine), *P. taeda* L. (loblolly pine), and *P. palustris* M. (longleaf pine) (Miller 1960, Bethune 1963 (circles on map), Frost 1963,1964, Miller and Wilson 1964, Kimball 1965).

In the West Indies and Central America, Miller reported *R. subtropica* from Pinar del Rio Province, Cuba (1960), and British Honduras (1965), now Belize, attacking *P. tropicalis* Morelet (tropical pine) and *P. caribaea* Morelet (Caribbean pine), respectively.

The objectives of this survey were to determine the best location for studying the biology of *R. subtropica* and to determine the distribution and abundance of *R. subtropica* in Florida.

Methods

From January 1971 until July 1974, J. R. McGraw and R. C. Wilkinson made collections, and solicited collections and collection records of *R. subtropica*.

R. subtropica larvae and pupae collected by and sent to J. R. McGraw and R. C. Wilkinson are deposited in the Florida State Collection of Arthropods, Gainesville.

R. subtropica adults which emerged from pupae collected by J. R. McGraw and G. W. Berisford in slash pine shoots at Glades County, Florida, are located at the Department of Entomology, University of Georgia, Athens (G. W. Berisford, personal communication).

Results

R. subtropica was collected in Alachua, Clay, Dixie, DeSoto, Flagler, Franklin, Glades, Hardee, Highlands, Lake, Madison, Putnam, St. Johns, Sarasota, Sumter, Taylor, and Volusia Counties, Florida, attacking *P. elliottii elliottii* and *P. palustris* by J. R. McGraw and R. C. Wilkinson. R. C. Wilkinson also collected *R. subtropica* from Sugarloaf Key, an insular component of Monroe County, Florida, and the most southern site on which pine (*P. elliottii densa*) grows naturally in the United States. R. C. Wilkinson collected and preserved larvae in Freeport, Grand Bahama Island, attacking *P. caribaea*.

Cooperative Research in Forest Fertilization (CRIFF) program Technical Representatives, H. E. Johstono and B. Poole, collected and supplied *P. elliottii elliottii* shoots containing *R. subtropica* from CRIFF A-Series test plots located in Bay, Franklin, Madison, and Taylor Counties, Florida, and Clinch County, Georgia.

R. subtropica specimens identified for the Division of Plant Industry (DPI), Florida Department of Agriculture and Consumer Services, were from *P. thunbergiana* Franco (Japanese black pine) nursery stock in Dade, Hillsborough, and Volusia Counties, Florida.

E. P. Merkel (personal communication) provided collection records of *R. subtropica* from Collier County, Florida, attacking *P. elliottii elliottii* and *P. elliottii densa*, and from south Georgia attacking *P. palustris*.

G. D. Hertel (personal communication) provided collection records from Baker, Columbia, Dixie, Nassau, Taylor, and Union Counties, Florida, of *R. subtropica* attacking *P. elliottii elliottii* in his research plots.

Discussion

R. subtropica was reported throughout coastal southeastern United States, the West Indies, and Central America.

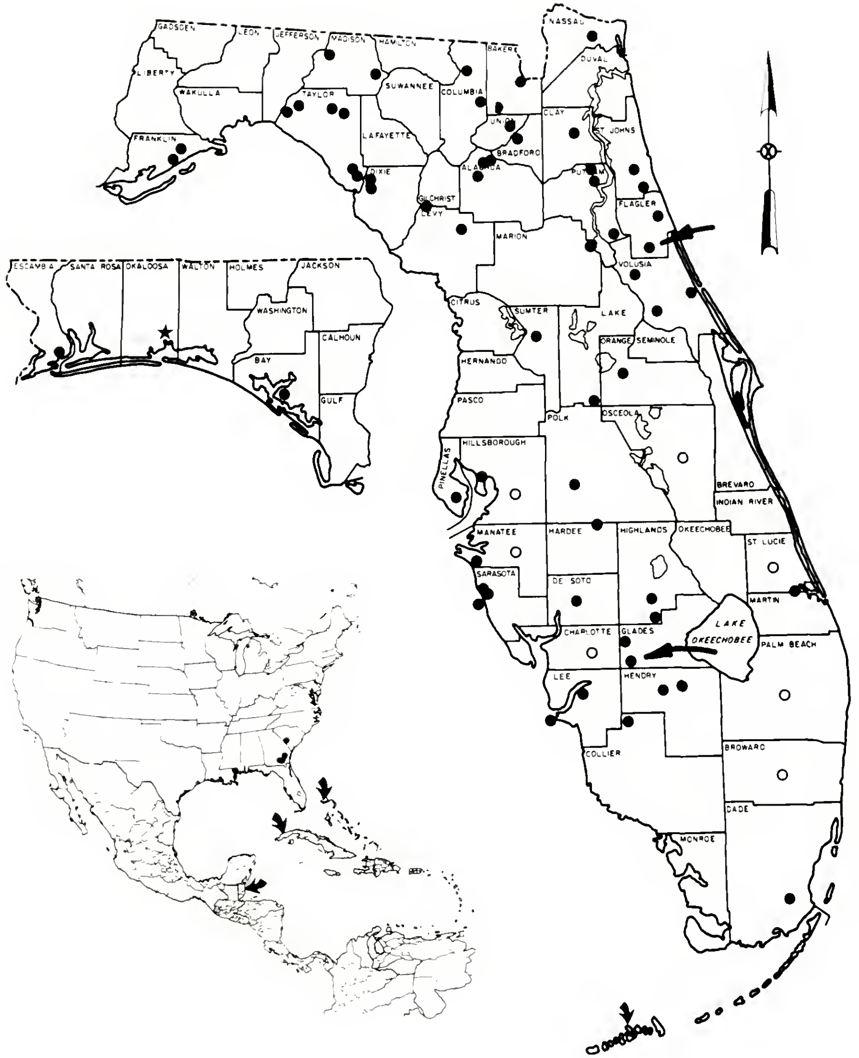
R. subtropica could be native to Central America and the West Indies and could have migrated or have been introduced into the southeastern United States.

Summary

This survey reported collections of *R. subtropica* attacking pines in southeast Georgia, on Grand Bahama Island, on Sugarloaf Key (the most southern site pines grow in the United States), and throughout Florida (Figure 2). *R. subtropica* was most abundant in the southern and coastal areas of Florida. Research plots for studying the biology of *R. subtropica* were established in a bedded 2-year-old *P. elliottii elliottii*[1] plantation located in southwestern Glades County, Florida, in the vicinity of Lykes Fire Tower (Sections 17, 18, and 20, Township 42 South, Range 29 East).

[1]Future references to slash pine, whether in north Florida or south Florida, mean *P. elliottii elliottii* and references to south Florida slash pine mean *P. elliottii densa*.

Figure 2. *R. subtropica* collection sites as determined from literature, personal communications, and collections; with circles denoting the south Florida counties, not exact sites, where Bethune (1963) worked, a star denoting type-locality, and arrows denoting the exact locations of J. R. McGraw's study plots in Flagler and Glades Counties.


NASSAU
GADSDEN
LEON
JEFFERSON
MADISON
HAMILTON
BAKER
DUVAL
LIBERTY
SUWANNEE
COLUMBIA
WAKULLA
TAYLOR
CLAY
UNION
ST JOHNS
FRANKLIN
LAFAYETTE
BRADFORD
ALACHUA
PUTNAM
DIXIE
GILCHRIST
FLAGLER
LEVY
MARION
ESCAMBIA
SANTA ROSA
OKALOOSA
WALTON
HOLMES
JACKSON
VOLUSIA
CITRUS
WASHINGTON
SUMTER
LAKE
CALHOUN
ORANGE
SEMINOLE
BAY
HERNANDO
GULF
PASCO
POLK
OSCEOLA
HILLSBOROUGH
PINELLAS
BREVARD
INDIAN RIVER
MANATEE
HARDEE
HIGHLANDS
OKEECHOBEE
ST LUCIE
SARASOTA
DE SOTO
MARTIN
CHARLOTTE
GLADES
LAKE OKEECHOBEE
PALM BEACH
LEE
HENDRY
COLLIER
BROWARD
DADE
MONROE

CHAPTER IV

THE NUMBER OF LARVAL INSTARS OF *RHYACIONIA SUBTROPICA*

Background

During initial studies of the biology of *R. subtropica*, knowledge of the number of larval instars was required for identifying where each instar fed on the slash pine host plant. Larval head capsules were measured to resolve the number of larval instars.

Methods

Biweekly from August 1972 to October 1973, slash pine shoots attacked by *R. subtropica* were collected in Glades County, Florida. Larvae were removed from the shoots, individuals were positioned on a small mound of 10% boric acid ointment in a BPI dish containing 70% ethanol, and maximum head widths were determined with the aid of an ocular micrometer mounted in a binocular dissecting microscope (magnification 40x).

The frequency distribution plot of head widths was analyzed by visual inspection. Instar means were calculated and χ^2 analysis (Snedecor and Cochran 1967) was used to compare

the goodness-of-fit of the observed calculated means to the means predicted by Dyar's rule (1890).

Results

The distribution of 1,635 head widths produced a histogram with 5 major peaks and 2 secondary peaks in the 4th and 5th instars (Figure 3). Chi-square analysis showed that the means predicted by Dyar's rule for the first 3 instars were highly correlated with the observed means (Table 1).

Discussion

The presence of 5 larval instars in *R. subtropica* corresponded to the number of larval instars in *R. frustrana* (Fox et al. 1972) and also conformed to Dyar's (1890) rule of geometric progression.

Summary

R. subtropica has 5 larval instars which conform to Dyar's (1890) rule of geometric progression. The means predicted by Dyar's (1890) rule for the first 3 larval instars were highly correlated with observed means for the first 3 larval instars of *R. subtropica*.

Figure 3. Distribution of *R. subtropica* larval head widths, with means (above) and ranges (below) of the 5 larval instars (n = 1,635 larvae).

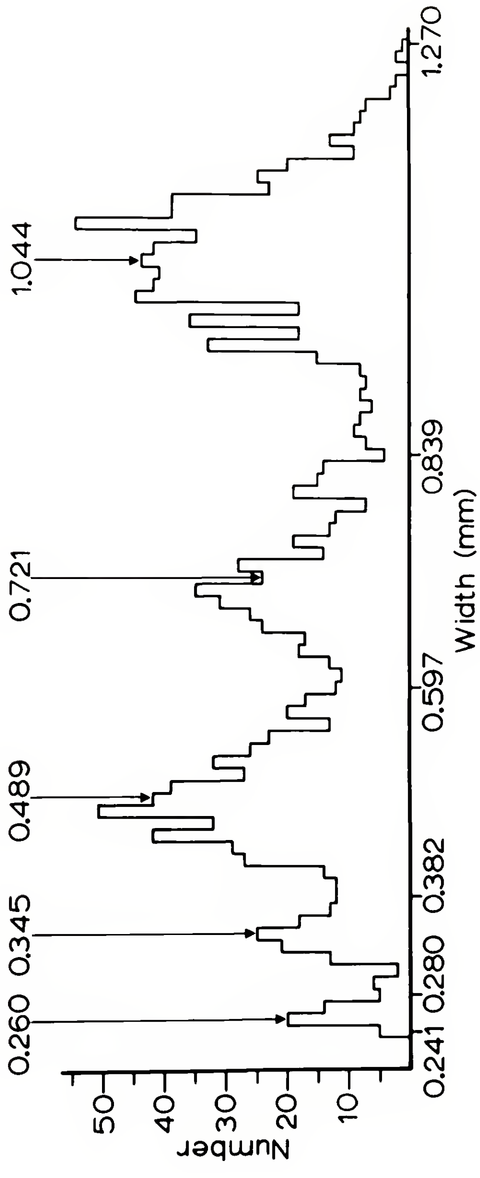

0.260
0.345
0.489
0.721
1.044
0.241
0.280
0.382
0.597
0.839
1.270
Width (mm)
Number
50
40
30
20
10

Table 1

Chi-Square Comparison of the Observed and Predicted Larval Head Width Means (mm) for the 5 Larval Instars of *R. subtropica*

	INSTAR				
	1	2	3	4	5
OBSERVED $\bar{x}$	0.260	0.345	0.489	0.721*	1.044*
s	0.01	0.02	0.05	0.06	0.08
n	45	109	460	344	677
PREDICTED $\bar{x}$	0.237	0.344	0.498	0.721*	1.044*
RANGE	0.241-0.280	0.292-0.382	0.394-0.597	0.610-0.839	0.851-1.270

*Dyar's ratio = 0.691; $\chi^2 = 0.173 < \chi^2_{0.975} = 0.22$.

CHAPTER V

SECONDARY SEX STRUCTURES AND SEX-ASSOCIATED SIZE DIFFERENCES OF LARVAL HEAD CAPSULES OF *RHYACIONIA SUBTROPICA*

Background

The investigation of larval head widths of *R. subtropica* showed the presence of 5 larval instars, corresponding to the number of larval instars in the smaller *R. frustrana* (Fox et al. 1972). In the larger *R. buoliana*, the uncertainty of the number of larval instars (possibly 6) is apparently complicated by a sex-associated difference in later stadia head widths (Friend and West 1933, Miller and Neiswander 1955, Pointing 1963).

Previous larval instar investigations by Drooz (1965) on *Ennomos subsignarius* (Hübner) (Lepidoptera:Geometridae), the elm spanworm, and by Wilkinson (1971) on *Neodiprion merkeli* Ross (Hymenoptera:Diprionidae), the slash-pine sawfly, showed that sex-associated size differences existed in the head widths of last stage larvae. Drooz correlated larval head width and sex by measuring a living individual larva's head capsules and by sexing its adult. Wilkinson found paired secondary sex structures (invaginated plates) he named "ovipositor bud plates" on the 8th and 9th abdominal larval sternites diagnostic of the female sex. Wilkinson studied

the metamorphosis of living larvae possessing the secondary sex structures to correlate the function and the sex associated with the structures. In 1967, Nielsen and Bohart reported structures on the 9th abdominal sternite of larvae (principally male) of certain wild bee species (Hymenoptera: Apidae). These latter authors did not investigate head widths or the number of larval instars.

The pupal sex-determining characters of *R. subtropica* are on the 8th and 9th abdominal sternites (Yates 1969).

The objectives of this investigation were to (1) locate and characterize any secondary sex structures present on 4th and 5th instar *R. subtropica* larvae, (2) correlate the presence or absence of these larval structures with pupal sex, and (3) describe possible sex-associated size differences in 4th and 5th instar larval head capsules.

Methods

Location of Secondary Sex Structures in Larvae

Preserved 4th and 5th instar *R. subtropica* larval integuments were split longitudinally along the dorsal mid-line, debrised, mounted in Hoyer's solution on microslides under 20 mm square coverslips, cleared, examined microscopically, illustrated, and photographed at 100x magnification.

Correlation of Pupal Sex with Larval Structures

R. subtropica larvae were collected 30 August 1972 in slash pine shoots in Glades County, Florida, removed from the

shoots, asphyxiated in a cool-water bath, and examined microscopically (magnification 60x) to determine the presence or absence of the structures located on the 8th and 9th abdominal sternites. The larvae then were revived and placed between moistened No. 2 Whatman filter paper disks in individual cups containing an aseptic agar medium (Batcheler and Emmel 1974) to await pupation. After pupation, the pupae were sexed according to Yates' (1969) technique to determine if the structures were associated with one sex.

Sex-Associated Size Differences in 4th and 5th Larval Instar Head Capsules

The 4th and 5th larval instars measured in the larval instar study were sexed according to the presence or absence of the secondary sex structures (magnification 60x) and head widths were correlated with sex. Larvae with damaged heads or missing abdominal segments were discarded.

The frequencies of the sexed 4th and 5th larval instar head widths were plotted separately and then superimposed. Separate male and female means were calculated for both the 4th and 5th larval instars. Student's *t*-test (Snedecor and Cochran 1967) was used to compare the 5th stage non-sexed (observed), male, and female means. Using Dyar's rule (1890) and both sets of sexed 4th and 5th larval instar means, two hypothetical series of means for the first 3 larval instars were calculated. The two hypothetical series of means were compared by χ^2 analysis to determine the goodness-of-fit to the observed non-sexed larval instar means.

Results

Location of Secondary Sex Structures in Larvae

Paired elliptical, aspinulose areas, surrounding transverse integumental folds, laterad of each V_1 seta and between the V_1 and SV setae were found on the 8th and 9th abdominal sternites of *R. subtropica* larvae (Figure 4). These 4 aspinulose areas were strongly similar and were not present on all 4th and 5th larval instars (Figure 5).

Correlation of Pupal Sex with Larval Structures

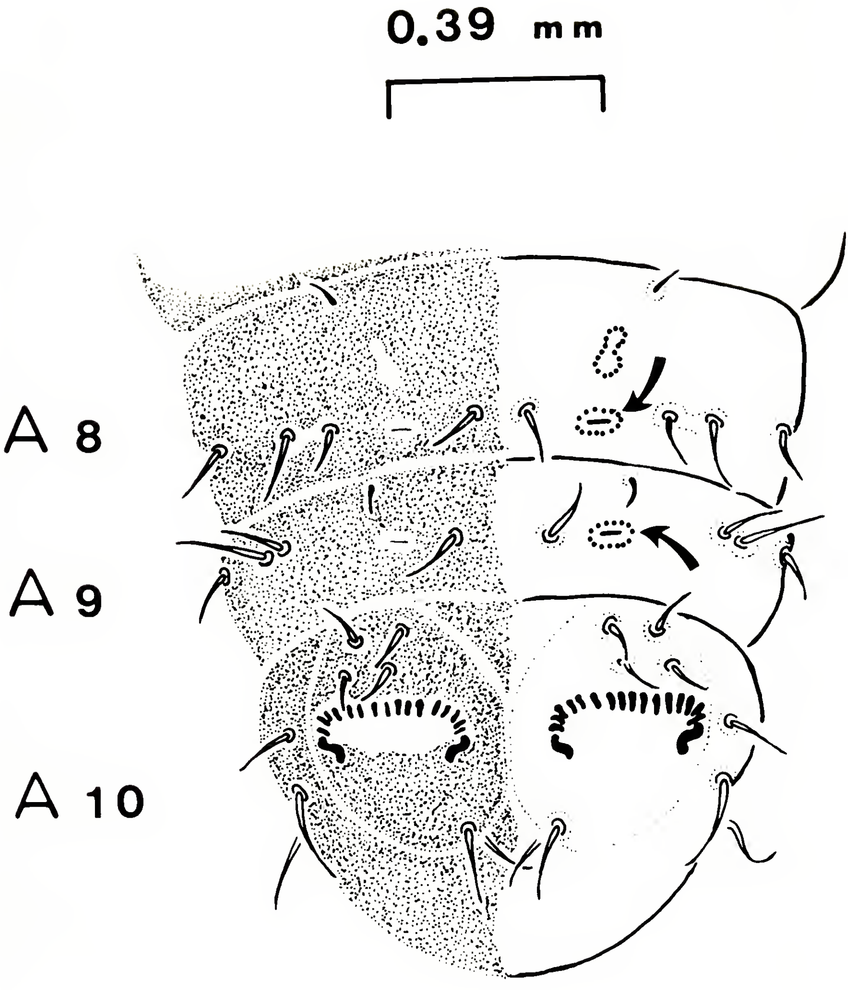
Larvae without the paired elliptical, aspinulose areas surrounding the transverse integumental folds on the 8th and 9th sternites (Figure 5A) produced only male pupae; and larvae with the paired structures (Figures 4 and 5B) produced only female pupae (Table 2), verifying only female larvae bore the secondary sex structures.

Sex-Associated Size Differences in 4th and 5th Larval Instar Head Capsules

Frequency plots of segregated male and female 4th and 5th larval instar head widths produced bimodal distributions for each stadium (Figure 6, top). The superimposed histogram showed the exact overlap between the ranges of the 4th instar females and 5th instar males. Twelve 4th stage female larvae were involved in this overlap and were considered 5th instars in the non-sexed data, calculations, and histogram (Table 3 and Figure 6, bottom).

Student's *t*-test comparisons between non-sexed, male,

Figure 4. Diagrammatic sketch of the 8th, 9th, and 10th abdominal sternites of a 5th stage *R. subtropica* female larva denoting (arrows) the location and characteristics of the secondary sex structures.



0.39 mm
A 8
A 9
A 10

Figure 5. Photomicrographs of 8th abdominal sternites of 5th stage *R. subtropica* larvae.

A. Male larva without a secondary sex structure.

B. Female larva with a secondary sex structure (arrow pointing anteriorly toward secondary sex structure).

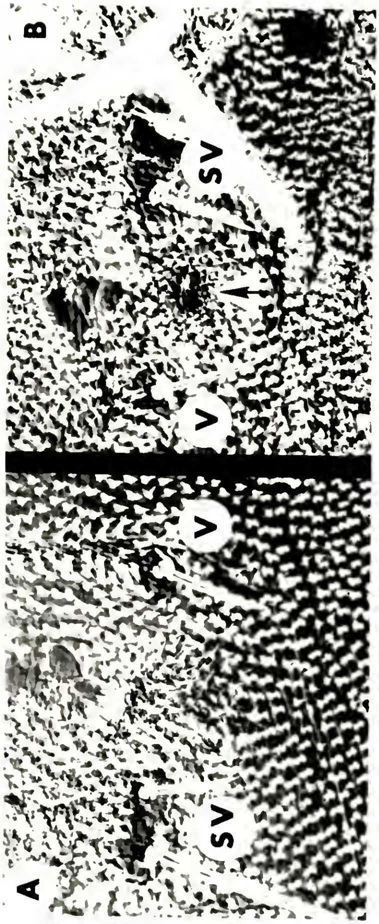

A
SV
V
V
SV
B

Table 2

Correlation of Pupal Sex with Presence (Female) or Absence (Male) of Secondary Sex Structures in *R. subtropica* Larvae

	LARVAE			PUPAE
	SEX PREDICTED	DIED	PUPATED	SEX VERIFIED
		(number)		
MALES	29	2	27	27
FEMALES	24	4	20	20

Table 3

Chi-Square Comparisons of Observed (Non-Sexed) and Male and Female Predicted Larval Head Width Means (mm) for the 5 Larval Instars of *R. subtropica*

	INSTAR				
	1	2	3	4	5
OBSERVED $\bar{x}_{NON-SEXED}$	0.260	0.345	0.489	0.721*	1.044*
$NUMBER_{NON-SEXED}$				344	677
PREDICTED $\bar{x}_{♂}$	0.242	0.343	0.491	0.703**	1.006**
$NUMBER_{♂}$				275	355
$RANGE_{♂}$				0.610-0.839	0.851-1.143
PREDICTED $\bar{x}_{♀}$	0.264	0.378	0.538	0.767***	1.095***
$NUMBER_{♀}$				81	310
$RANGE_{♀}$				0.648-0.876	0.928-1.270

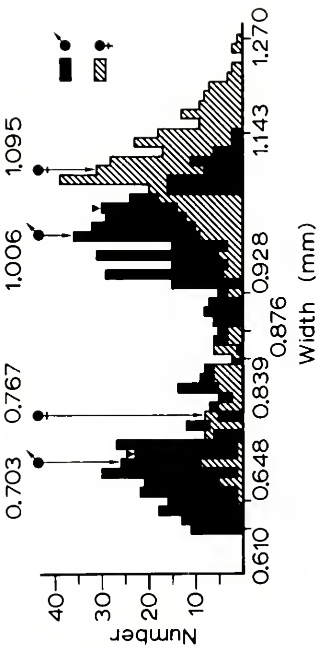
*Dyar's $ratio_{non-sexed} = 0.691$.

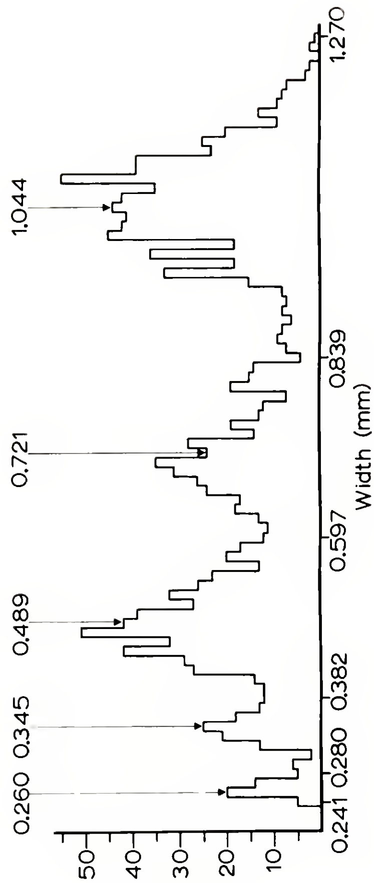
**Dyar's $ratio_{♂} = 0.699$; $\chi^2 = 0.139 < \chi^2_{0.975} = 0.22$.

***Dyar's $ratio_{♀} = 0.701$; $\chi^2 = 0.809 < \chi^2_{0.75} = 1.21$.

Figure 6. (Top) Superimposed distributions of 4th and 5th stage *R. subtropica* male ($n = 630$) and female ($n = 391$) larval head widths (mm).

(Bottom) Distribution of non-sexed head widths (mm) of the 5 larval instars of *R. subtropica* ($n = 1,635$). Means are indicated above the histograms and ranges below.


0.703
0.767
1.006
1.095
Number
40
30
20
10
0.610
0.648
0.839
0.876
0.928
1.143
1.270
Width (mm)


0.260
0.345
0.489
0.721
1.044
Number
50
40
30
20
10
0.241
0.280
0.382
0.597
0.839
1.270
Width (mm)

and female 5th larval instar means showed that the 3 means were significantly different.

Chi-square analysis showed that the hypothetical first 3 larval instar means predicted from the male Dyar's ratio were more closely correlated with the observed (non-sexed) means than the hypothetical means predicted from the female Dyar's ratio (Table 3).

Discussion

The location and appearance of the paired transverse integumental folds found on the 8th and 9th abdominal sternites of female *R. subtropica* larvae were similar but not identical to the transversely invaginated ovipositor bud plates of female *N. merkeli* sawfly larvae (Wilkinson 1971) or the secondary sex structures of certain wild bee larvae (Nielsen and Bohart 1967).

The secondary sex structures were evident without the use of stains, but high magnification (60x) and excellent lighting were required. The use of stains (Nielsen and Bohart 1967) is advised when light colored or small larvae are studied.

A careful microscopic examination of the 8th and 9th sternites of unstained, preserved last larval instars of *R. frustrana*, *Malacosoma americanum* (F.), *M. disstria* (Hbn.), *Porthetria dispar* (L.), *Ceratomia catalpae* (Bdv.), and *Tetralopha robustella* Zell. revealed that a portion of the specimens of each of these lepidopterous forest pest species also possessed structures similar in appearance and location

to those structures found on female *R. subtropica* larvae and the species discussed in Wilkinson (1971) and Nielsen and Bohart (1967). Additional studies are required to determine the sex of the larvae of these 7 additional species which possessed the paired structures. Such structures could be common in other lepidopterous larvae.

Larval mortality during the sex verification experiment probably was attributable to (1) injury to larvae during removal from slash pine shoots, (2) cross-contamination with unknown pathogenic organisms during asphyxiation of larvae in common cool-water bath, (3) injury to larvae being handled during sexing procedure, and (4) use of penultimate larval instars which starved to death before pupation.

The inclusion of 12 female 4th larval instars in the 5th larval instar non-sexed head width data and calculations could not have been known, nor the exact extent of the overlap of 4th and 5th stadia determined without sexing the 4th and 5th larval instars.

The Student's t-test comparisons of the male and female 5th larval instars head width means proved the head capsules of the female *R. subtropica* 5th larval instar were significantly larger than those of the 5th larval instar males. Chi-square analyses of the male, female, and non-sexed Dyar's ratios were not widely dissimilar; since the size of male and female 5th larval instar head capsules were not the same and the 4th and 5th larval instar means used as starting points for Dyar's geometric progression were not equal, neither the

male nor the female ratio predicted the first 3 larval instar means as well as the non-sexed ratio.

The unbalanced sex ratio in the 4th stadium was caused by my inability to discern microscopically the secondary sex structures, and not by the lack of female larvae. If female larvae develop faster than male larvae, which is not the case, the frequency and number of collections during this 13-month study would have tended to have equalized the 4th larval instar's sex ratio. Also, the balanced 5th larval instar sex ratio suggested that the number of 4th instar females probably equaled the males but the failure to discern secondary sex structures resulted in larvae being identified as males. Care should be taken and caution applied when sexing *R. subtropica* 4th larval instars.

The ability to sex 4th and 5th stadium larvae elucidated the instar limits; determined the exact instar overlap; determined the larval sex ratios; eliminated secondary peaks in the non-sexed histogram of head widths; produced bimodal distributions within the 4th and 5th instars; eliminated skewed, non-sexed distributions in the non-sexed histogram of head widths; and proved a sex-associated size difference in head capsules of 4th and 5th instar *R. subtropica* larvae. The use of larval sex also supported the presence of 5 larval instars. In addition to the above uses and the uses mentioned by Neilsen and Bohart (1967) and Wilkinson (1971), secondary sex structures would be useful for identifying living female larvae and establishing sex ratios in behavioral, ecological, biological control, and pest management studies.

Dyar's rule of geometric progression has been applied many times to estimate the number and mean head widths of larval instars of various lepidopterous and hymenopterous species. Predictions calculated by using Dyar's rule have been accepted about as many times as they have been rejected. Probably the major factors contributing to the rejection of predictions calculated by using Dyar's rule were (1) the small number of head capsules measured, (2) large differences in the widths of the head capsules of penultimate and ultimate stage male and female larvae, and (3) an unbalanced sex ratio in the penultimate and ultimate instars causing their head width means, from which Dyar's ratio is calculated, to be skewed. When a person has studied the biology of a particular species, measured numerous larval head capsules of all instars (with or without segregating sizes according to sex), and constructed a clear frequency distribution of the larval head widths, the Dyar's ratio and geometric progression calculated should be used as tools for gauging the natural sequential size increases of larval head capsules, not used to set the progression.

Summary

The 4th and 5th *R. subtropica* female larval instars had paired secondary sex structures on their 8th and 9th abdominal sternites. The 5th larval instar female head capsules were statistically larger than the head capsules of the 5th larval instar males.

CHAPTER VI

SEASONAL HISTORY OF *RHYACIONIA SUBTROPICA* IN SOUTH FLORIDA

Background

The seasonal history of *R. subtropica* was studied in slash pine plantations in south Florida because (1) this tip moth was more abundant there than in other areas of Florida, (2) *R. frustrana* and *R. rigidana* did not occur in south Florida, and (3) previous pitch canker disease studies, which incriminated *R. subtropica*, were conducted near La Belle, Hendry County, in south Florida (Anonymous 1958, Bethune and Hepting 1963).

Methods

Biweekly from August 1972 to October 1973 and periodically thereafter until July 1974, slash pine shoots attacked by *R. subtropica* were collected from 2- and 3-year-old bedded plantations in the vicinity of Lykes fire tower, north of La Belle, in southwest Glades County, Florida (Sections 17, 18, and 20, Township 42 South, Range 29 East).

Larvae were removed from shoots and preserved in 70% ethanol. Head capsules were measured for instar determination. Pupae removed from shoots were placed in individual

rearing cups containing an aseptic agar medium (Batcheler and Emmel 1974) and were held until adult emergence in a rearing room at 27 ± 2°C with north light for exposure to natural photoperiod (Gainesville, Florida).

Observations on the seasonal height growth (flushing) of slash pine were also made in Glades County, Florida.

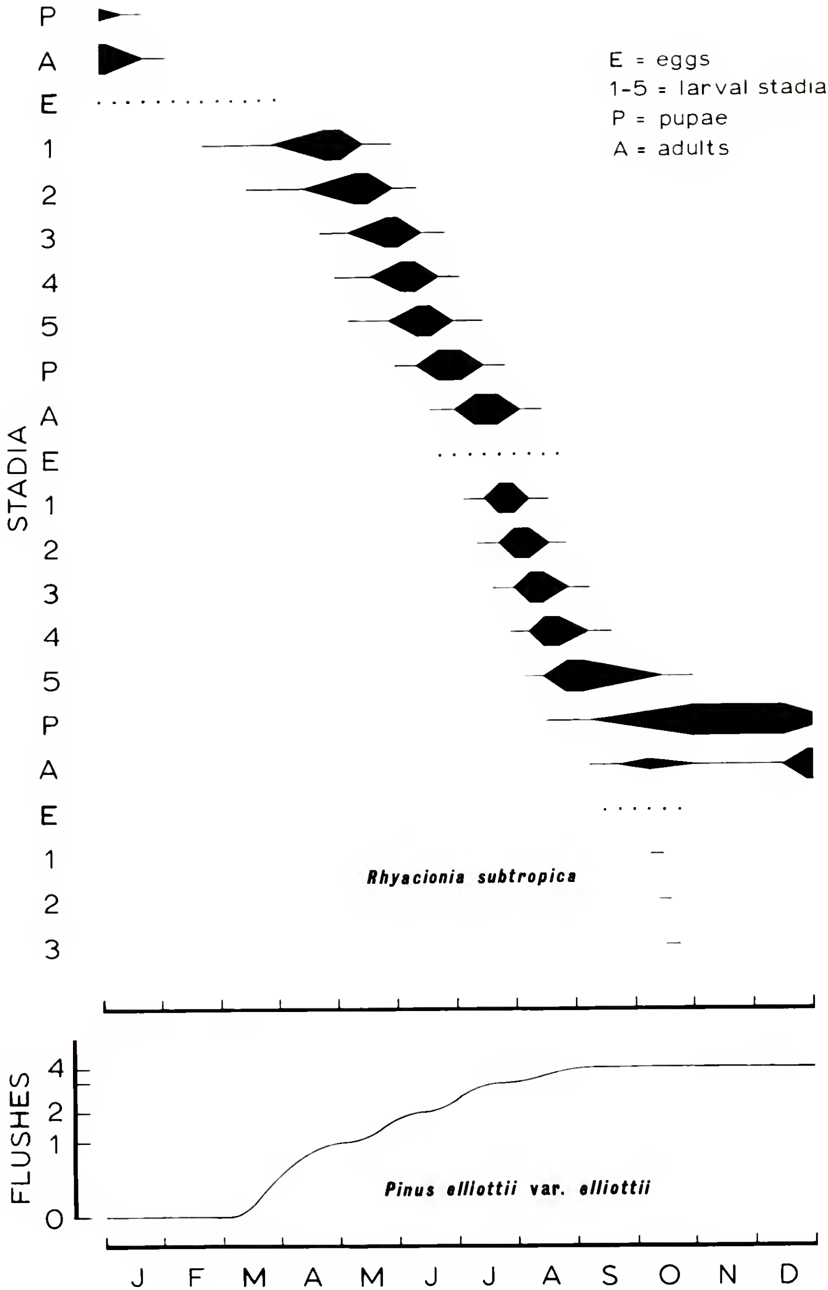
Results

The seasonal history of *R. subtropica* and the multinodal growth (flushing) pattern of slash pine in south Florida during 1972 and 1973 are illustrated in Figure 7. *R. subtropica* was bivoltine with a partial 3rd generation during the fall. *R. subtropica* pupae "overwintered" in the field in south Florida; however, once the pupae were taken into the laboratory, adult emergence soon occurred. *R. subtropica* eggs were never found, and the probable occurrence of eggs in Figure 7 was based on the relative abundance of adults and 1st stage larvae.

R. subtropica collected by R. C. Wilkinson on 23 June 1973 at Freeport, Grand Bahama Island, were synchronized with the Glades County population.

The seasonal height growth (flushing) of slash pine in Glades County began during early March, with the majority of the trees attaining 3 flushes of growth and others attaining 4-6 flushes. After first flush initiation, subsequent flushing was not synchronized among trees.

Figure 7. Seasonal history of *R. subtropica* and multinodal growth pattern (flushing) of slash pine (*P. elliottii elliottii*) in south Florida during 1972 and 1973.



E = eggs
1-5 = larval stadia
P = pupae
A = adults
STADIA
P
A
E
1
2
3
4
5
P
A
E
1
2
3
4
5
P
A
E
1
2
3
Rhyacionia subtropica
FLUSHES
4
2
1
0
Pinus elliottii var. elliottii
J F M A M J J A S O N D

Needle elongation on each flush of growth occurred from the base upward and was completed before the next flush elongated. In the spring of 1974, 1st flush needles did not elongate or elongated from the apex downward, apparently due to a severe spring drought followed by early summer torrential rains.

Discussion

The partial September-October emergence observed in south Florida possibly represents a third generation during some years. Second generation *R. subtropica* pupae collected in north Florida also exhibited a tendency for a partial third generation.

The seasonal multinodal growth characteristic of slash pine in south Florida was similar to the multinodal growth characteristic of slash pine in north Florida (Kaufman 1965, Pritchett and Smith 1970), but periods of shoot elongation (flushing) varied between slash pines growing in north and south Florida.

In south Florida, the seasonal histories of both *R. subtropica* and slash pine exhibited intersynchronization in the (1) initiation of 1st generation 1st instar larval development and 1st flush shoot elongation, (2) completion of 1st generation larval feeding and attainment of a majority of the height growth before 21 June, and (3) termination of 2nd generation larval development and host plant growth. The

asynchronization of flushing from June-August continuously provided new and undamaged host plant tissues suitable for tip moth oviposition and early larval instar development.

Summary

R. subtropica was bivoltine in south Florida with a partial 3rd emergence in September and October. The seasonal histories of both *R. subtropica* and slash pine exhibited intersynchronization in south Florida.

CHAPTER VII

LARVAL FEEDING OF *RHYACIONIA SUBTROPICA*

Background

Yates (1967a) reported the location of *R. frustrana* and *R. rigidana* larval feeding on loblolly pine shoots during April and May. He found that 1st, 2nd, and 3rd larval instars fed on the exterior of shoots (i.e., injury) and that 4th larval instars began internal boring (i.e., damage) after such shoots were almost completely elongated.

Yates (1966) has defined injury and damage caused to loblolly pine and slash pine by larval feeding of *R. frustrana* and *R. rigidana* as follows: "injury refers to needle and external shoot feeding by the larvae; damage refers to internal feeding which causes distortion or death of the shoot or bud." Yates (1960) also divided damage into deformation of the tree (main stem), retardation or loss of height growth, and reduction of cone crops. Heikkenen (1960) outlined deformations of the main stem of red pine as crooks, forks, pruning, brushing, and spike-tops. Miller (1967) defined Heikkenen's (1960) deformation categories as follows: (1) "A crook is a departure of the stem from straightness," (2) "A fork . . . occurs when two or more shoots closely compete for dominance," (3) "Pruning . . . is a reduction in the normal

number of branches at a whorl due to killing of one or more buds or shoots," (4) "Bushing . . . is an increase in the normal number of branches at a whorl due to fascicle buds which appear after all or most of the original leader buds or shoots are killed," and (5) "Spike-tops . . . are dead leaders killed back so that fascicle buds form well below the tip or do not form at all."

The objectives of this *R. subtropica* larval feeding study were (1) to determine which host tissues the 5 larval instars ate, and (2) to determine how the various types of feeding affected slash pine growth.

Methods

Tissues Eaten

The descriptions of *R. subtropica* larval feeding were made from laboratory observations of field-collected material, and the botanical terminology used to describe the slash pine structures and tissues which *R. subtropica* larvae ate is that of Doak (1935).

Effect on Slash Pine Growth

The definitions and categories of Yates (1960, 1966), Heikkenen (1960), and Miller (1967) were used to report laboratory examinations and field observations made in both Flagler and Glades Counties. *R. subtropica* larval feeding injury and damage and the effect of the feeding on slash pine growth are reported.

Results

Tissues Eaten

First stage *R. subtropica* larvae fed (1) on young scales and fascicles on elongating young vegetative long shoots, (2) inside the fascule sheath on the bases of unelongated or partially elongated secondary needles of a fascicle, (3) inside one elongating or completely elongated secondary needle of a fascicle, and (4) on juvenile needles of elongating young vegetative dwarf shoots (Figure 8).

Second stage *R. subtropica* larvae (1) fed on young scales and fascicles on elongating young vegetative long shoots, (2) fed in the groove between scales subtending a fascicle, (3) bored into the side or keeled-ridge of a scale subtending a fascicle and fed on the internal tissues under the fascicular insertion, and (4) fed on juvenile needles of elongating young vegetative dwarf shoots. During the second type of feeding described above, the larva exited the scale through the "entrance" hole before boring into another scale; consequently, the internal scale feeding sites were not connected by internal tunnels (Figure 9).

Third stage *R. subtropica* larvae initiated boring into the vascular tissues of the elongated vegetative long shoots.

Fourth and 5th stage *R. subtropica* larvae extended the tunneling initiated by 3rd stage larvae, tunneled up the shoot into the apical bud, and consumed the bud's tissues.

Figure 8. First stage *R. subtropica* larval feeding injury to slash pine.

A. Dead partially elongated secondary needles with a larval exit hole in the fascule sheath.

B. Elongated secondary needles with larval mining and the distal portion of the upper needle dead.

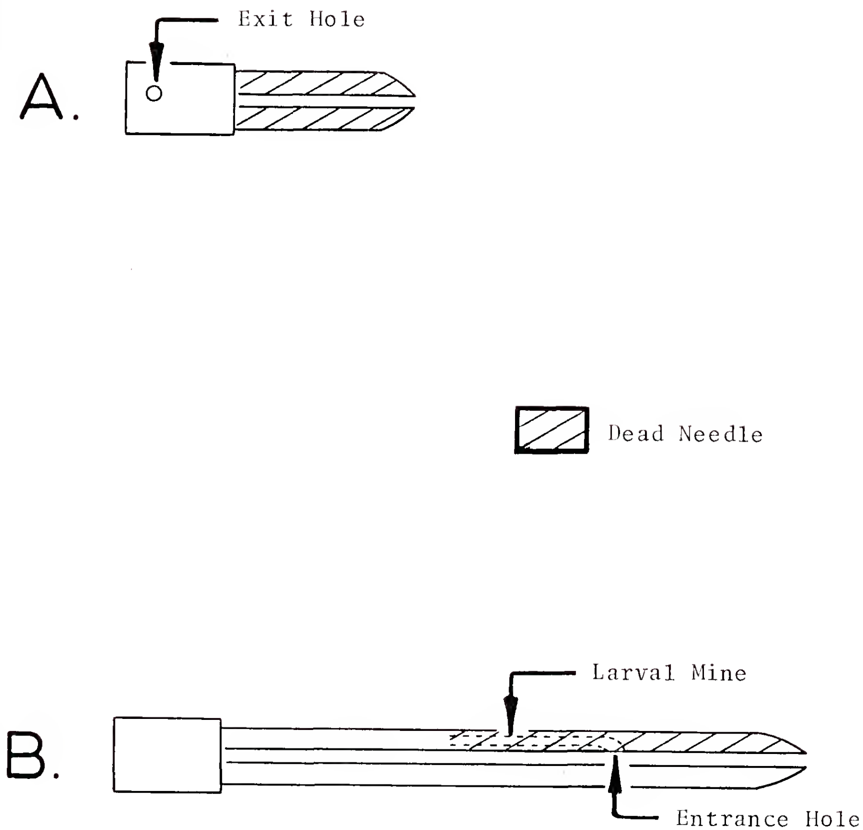

Exit Hole
A.
Dead Needle
Larval Mine
B.
Entrance Hole

Figure 9. Second stage *R. subtropica* larval feeding injury on slash pine.

A. A scale with an entrance and exit hole in the scale's keeled-ridge.

B. A cross-sectional view of a young vegetative long shoot with an arrow denoting the depth of larval feeding injury in the groove between scales. This particular *R. subtropica* larval feeding site was invaded by *Cecidomyia* sp. larvae (Diptera:Cecidomyiidae).


A


B

Effect on Slash Pine Growth

Injury occurred (1) when 1st stage *R. subtropica* larvae bored inside the fascule sheaths of unexpanded secondary needles, (2) when 1st stage *R. subtropica* larvae bored inside elongating or completely elongated secondary needles, and (3) when 2nd stage *R. subtropica* larvae bored into scales subtending fascicles. The result of injury was the death of all foliar tissues distal of the larval feeding.

Damage occurred (1) when 1st and 2nd stage *R. subtropica* larvae fed on young scale and fascicles on elongating young vegetative long shoots, (2) when 1st and 2nd stage *R. subtropica* larvae fed on juvenile needles of elongating young vegetative dwarf shoots, (3) when a single 3rd stage *R. subtropica* larva bored into a small diameter elongating vegetative long shoot, and (4) when one or more 3rd stage *R. subtropica* larvae bored into the vascular tissues of the vegetative long shoot, moulted, and the succeeding 4th and 5th instars continued to tunnel up the shoot and into the bud. The extent of damage (length of shoot killed) was directly proportional to the number of larvae feeding in a shoot.

Forking was the only deformity resulting from *R. subtropica* damage to slash pine shoots at Relay in north Florida.

Crooks, forks, bushing, and spike-tops were caused by *R. subtropica* damage to slash pine in Glades County in south Florida.

No definitive studies have been reported or were conducted to determine the extent of loss of height growth in slash pine caused by *R. subtropica* damage in either north or south Florida.

Reduction of cone crops of slash pine caused by *R. subtropica* damage was not studied. R. C. Wilkinson (personal communication) collected *R. subtropica* larvae feeding in male catkins on *P. elliottii densa* at Sugarloaf Key, Monroe County, Florida.

Discussion

The slash pine tissues eaten by the larval instars of *R. subtropica* were essentially the same tissues Yates (1967a) reported consumed when *R. frustrana* and *R. rigidana* larvae fed on loblolly pine. The generalizations Yates (1966) made concerning the larval feeding of *R. subtropica*, *R. frustrana*, and *R. rigidana* were valid.

Crooks, bushing, spike-tops, and reduction of height growth were not usually caused when *R. subtropica* larvae damaged slash pines growing in north Florida because (1) laterals rapidly expressed apical dominance and the main stem straightened within 1 to 3 years, and (2) *R. subtropica* did not frequently or repeatedly attack the new apical shoots of previously attacked trees. Yates (1966) concluded that *R. frustrana* and *R. rigidana* did not severely damage slash pines growing in central Georgia.

Crooks, forks, bushing, spike-tops, and reduction of height growth occurred when *R. subtropica* larvae damaged slash pines growing in south Florida because (1) slash pine was planted out of its natural range (Bethune 1963), and

(2) *R. subtropica* continually attacked new apical shoots of previously attacked trees. Bethune (1966) also reported *Rhyacionia* spp. repeatedly attacked and damaged slash pines in south Florida.

Cone crop damage caused by *R. subtropica* in slash pine seed orchards in the southeastern United States probably has not been as severe as *R. frustrana* cone crop damage in shortleaf pine seed orchards (Yates and Ebel 1972, Ebel and Yates 1974), and has not been studied because (1) investigators have just become aware of the potential conelet mortality that tip moth damage can cause, (2) most slash pine seed orchards are not located in south Florida where *R. subtropica* is most abundant, and (3) insect control practices are an integral component of seed orchard maintenance programs.

Summary

First and 2nd stage *R. subtropica* larvae fed on foliar tissues or tissues associated with foliar tissues. The 3rd, 4th, and 5th stage *R. subtropica* larvae tunneled into the internal tissues of vegetative long shoots and apical buds.

Injury was associated with 1st and 2nd stage *R. subtropica* larval feeding, with two exceptions.

Damage was caused by 3rd, 4th, and 5th stage *R. subtropica* larval feeding. *R. subtropica* damage did not usually produce deformities in slash pines grown in north Florida, but usually caused the deformity of slash pines growing in south Florida.

CHAPTER VIII

PUPAL BEHAVIOR, WEIGHT, AND SEX RATIO OF *RHYACIONIA SUBTROPICA*

Background

Pupation, the act of becoming a pupa, was initiated with the pre-pupation activities of mature 5th stage larvae of *R. subtropica* and terminated with the initiation of the pre-eclosion activities of the encased pharate *R. subtropica* adult.

The objectives of this study were to report (1) the activities of *R. subtropica* from the mature 5th stage larva to the pre-eclosion pharate adult, (2) pupal weight, and (3) the pupal sex ratio.

Methods

General

The descriptions of the pupation behavior of *R. buoliana* (Pointing 1961, 1963) were used as guides for observing the pupation activities of *R. subtropica*. The observations on pupation activities were made during the removal of *R. subtropica* instars from slash pine shoots collected in Glades County, Florida, from August 1972 through June 1974.

Pupation Sequence

On 31 August and 15 September 1972 the apical to basal sequence of pupae and larvae in 35 multiple-infested shoots was recorded. The arrangement in 25 shoots was reported. Shoots containing all pupae, all larvae, or single instars were not recorded.

The apical to basal pupal sex sequence in all shoots containing multiple pupae was recorded on 13 September 1973. Shoots containing a single pupae or all larvae were not recorded.

Pupal Weight

The pupae recovered on 31 August 1972 were also weighed and sexed. The mean weights of those male and female pupae which later issued adults were calculated.

Pupal Sex Ratio

The sex ratio before eclosion was calculated for 425 *R. subtropica* pupae collected during August and September, 1972, and 276 pupae collected during July 1974.

Results

Pre-pupation Behavior

R. subtropica 5th stage larvae constructed a silk-lined pupation chamber and prepared the pupal exit hole in the apical portion of the mined shoot. The pupal exit hole was at or in the vicinity of the point of fascicular insertion above

the fascicular scale. On multiple-infested shoots the exit holes were scattered along the apical portion of the mined shoot. *R. subtropica* pupal exit holes were not obvious or easily located.

Pupation Sequence

Pupation proceeded almost entirely from the apex toward the base in multiple-infested shoots (Table 4 and Figure 10) and the vertical sequence of pupal sex had no apparent pattern (Table 5).

Pupal Weight

The range of weights for 34 male pupae was 7.2-25.6 mg with a mean weight of 18.9 ± 0.13 mg ($\overline{x} \pm t_{0.05}\ s_{\overline{x}}$) and the range for 44 female pupae was 17.1-47.4 mg with a mean of 29.9 ± 0.08 mg.

Pupal Sex Ratio

The September 1972 pupal population (n = 425) was 44% females, and the July 1974 pupal population (n = 276) was 62.4% females. The total pupae of the 1972 and 1974 populations averaged 51.2% females before emergence. The sex ratio (♀:♂) considering all 701 pupae from 1972 and 1974 was 51:49 (ca. 1:1), without regard to adult emergence.

Variation of Pupal Color

Newly formed pupae were yellowish-brown. Thereafter, pupae darkened to become amber colored. Prior to eclosion, the amber pupae turned dark brown with the eyes, antennae,

Table 4

Apical to Basal Pupation Sequence of *R. subtropica* in 25 Multiple-Infested Slash Pine Shoots from Glades County, South Florida

APEX

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)
P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	L	L	L	L	L	L	P
P	P	P	P	P	P	P	P	P	P	L	L	L	L	L	L	L	L	L	L					L*
P	P	P	P	P	P	L	L	L	L	L	L	L												P
P	L	L	L	L	L	L				L	L	L												P
L	L	L								L	L													L
L	L									L														L
L																								
L																								
L																								

BASE

Note: P = pupa, L = larva.

*Indicates the only observed larva which occurred above a pupa.

Figure 10. Pupation sequence of *R. subtropica* instars (P = pupa, L = larva) in a slash pine shoot with dead, partially elongated secondary needles (N).

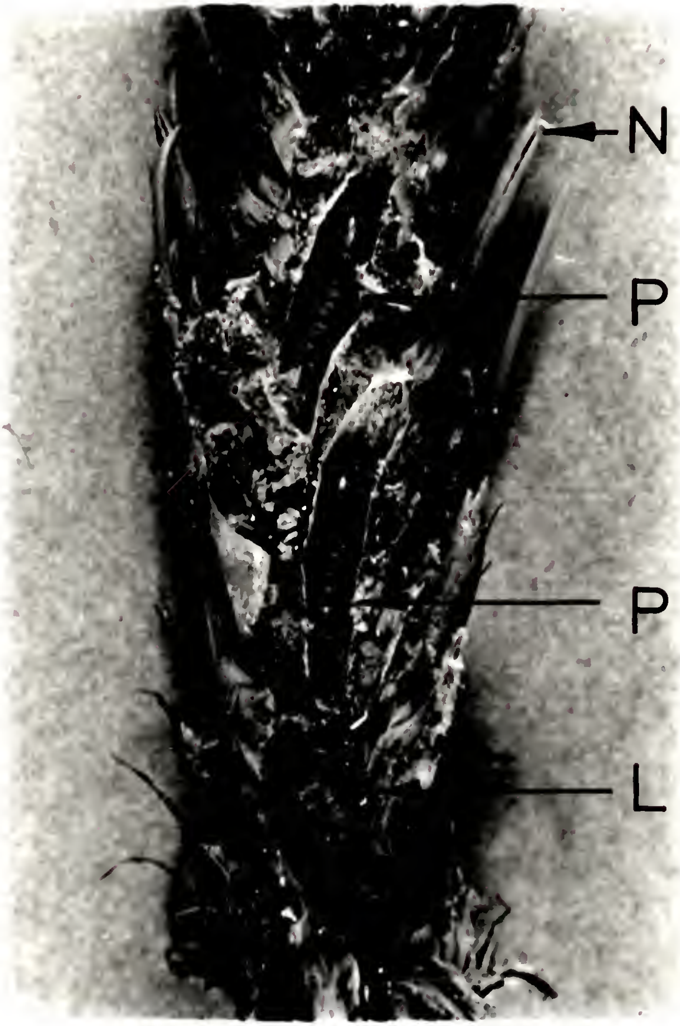

N
P
P
L

Table 5

Apical to Basal Arrangement of *R. subtropica* Male (♂) and Female (♀) Pupae in 12 Multiple-Infested Slash Pine Shoots from Glades County, South Florida

APEX

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
♂	♀	♀	♀	♀	♀	♀	♂	♂	♀	♂	♀
♂	♀	♂	♀	♂	♂	♀	♂	♀	♀	♀	♂
♀	♀	♂	♀	♂	♀	♂	♀	♂			
♂	♂	♀	♂	♀	♂	♂					
♀	♂	♀	♂								
♀	♂	♀									
♂	♂										
♂											

BASE

banded wings, and banded legs of the pharate adult becoming evident.

Duration of Pupal Stage

The duration of the pupal stage of individual pupae was not specifically studied, but in south Florida *R. subtropica* pupae were present from late August until February and from early June until late July (Figure 7).

Discussion

The mature 5th stage *R. subtropica* larvae which were head-up within the silk-lined pupal chamber had usually terminated feeding, and could be removed from the shoot and held for pupation to provide additional adults for laboratory investigations. Furthermore, since the mature 5th stage larvae can be sexed, these larvae could also provide a source of insects of known sex, age, and stage of physiological development for laboratory experiments.

The pre-pupation activities of 5th stage *R. subtropica* larvae were biologically similar to those activities of *R. buoliana* (Pointing 1963), but unlike *R. buoliana*, the *R. subtropica* pupal exit hole was not usually located in the wall of the bud.

The multiple infestations and pupations per shoot as observed for *R. subtropica* attacking slash pine in south Florida were also commonly observed for *R. frustrana* attacking loblolly pine shoots in north Florida. A single pupa per

bud was reported for *R. buoliana* (Pointing 1963).

The distribution of multiple *R. subtropica* larvae and pupae in shoots probably reflected termination of feeding activities and maturation of larvae. Based on the downward progression of pupation and the apparently random vertical distribution of pupal sex within a given shoot, apparently neither male nor female larvae required additional feeding time as a larva nor did either sex larva pupate first.

The *R. subtropica* pupal sex ratios fluctuated over time similarly to the reported (Pointing 1961) sex ratios of *R. buoliana* adults.

The pupal weights were reported for the use of future workers wishing to evaluate artificial diets developed for or used to rear *R. subtropica* larvae.

Summary

The mature 5th stage *R. subtropica* larvae constructed silk-lined pupal chambers in and prepared pupal exit holes along the apical portions of mined shoots. In multiple-infested shoots, pupation generally proceeded downwardly with an apparently random vertical distribution of pupal sex. The weight by sex, sex ratio, colors, and duration of *R. subtropica* pupae are also reported.

CHAPTER IX

EMERGENCE OF *RHYACIONIA SUBTROPICA*

Background

Emergence was considered to begin with pre-eclosion activities of the encased pharate *R. subtropica* adults and end with the activities immediately following eclosion and wing expansion of the moths. The objectives of this study were (1) to describe individual emergence activities and (2) to determine the daily pattern of emergence.

Methods

General

Accounts of emergence activities in *R. buoliana* (Pointing 1961, 1963; Green 1965) and *Dioryctria abietella* (Dennis and Schiffermüller) (Lepidoptera:Pyralidae) (Fatzinger and Asher 1971) were used as study guides.

R. subtropica pupae collected 31 August and 15 September 1972 in Glades County, Florida, were used to determine emergence times, and emergence patterns were analyzed by Batschelet's (1965) statistical methods. Mean times were calculated for wing inflation, wing drying, and complete wing expansion.

Emergence Cup Technique

R. subtropica pupae were removed from shoots, sexed according to Yates' (1969) technique, placed in individual clear plastic cups containing an aseptic agar medium (Batcheler and Emmel 1974) and capped with serially numbered lids, then held in a rearing room at 27 ± 2°C with north windows for exposure to natural photoperiod (Gainesville, Florida; see Table 6) until pharate adults developed. Pupae were examined daily under a dissecting microscope (magnification 20x) to determine the presence of pharate adults. The rearing room's overhead incandescent and fluorescent lamps were set for a 12-hour light:12-hour dark diel light cycle synchronized to come on after sunrise and go off before sunset.

Daily until emergence, cups containing pharate adults were removed from the darkened rearing room and placed out-of-doors on the south wide of the laboratory at least one hour before sunrise. The cups with non-emerged pupae were returned to the rearing room after 1100 hour Eastern Daylight Saving Time (EDST). The cups were again placed outside at least one hour prior to sunset and only returned to the darkened rearing room after sunset. These arrangements subjected the mature pupae to a natural sunrise, photoperiod, and sunset. During the night pupae were examined in the darkened rearing room with the aid of a battery-operated light dimmed with a red acetate film.

Times of adult emergence were recorded in hours and minutes EDST while times required for wing inflation and drying

were recorded to the nearest 1/4-minute.

Emergence Board Technique

Twenty shoots suspected of containing *R. subtropica* pupae were affixed vertically to a board as described by Pointing (1961) and Green (1965). This board and its affixed shoots were held and handled daily in the same manner as the emergence cups containing pharate adults. Activities of adults which eclosed from shoots were recorded in the same manner as for adults which eclosed in the emergence cups, but their sex was not determined.

The emergence board technique was used to simulate conditions as they might occur on vertical shoots in the field and to observe post-eclosion activities of moths under such conditions.

Emergence Recorder Technique

Pupae were taken to the Naval Stores and Timber Production Laboratory, USDA Forest Service, Olustee, Florida, and held in C. W. Fatzinger's (1970b) emergence recorder. The recorder was placed in an outdoor insectary to provide exposure to natural sunrise, photoperiod, and sunset. Otherwise, the recorder was operated and maintained as reported by Fatzinger (1970b).

The emergence recorder's charts were used to corroborate emergence observed in the previous tests and general emergence behavior in the absence of an observer.

Results

Pre-Eclosion Activities

The darkened *R. subtropica* pupae containing pharate adults held in emergence cups rotated their abdomens frequently and vigorously during the day and hours preceding emergence. Immediately prior to eclosion, the pupae held in emergence cups straightened, lengthened, and initiated posterior to anterior peristalsis.

Eclosion Activities

As the adult began to eclose, the head of the pupal case split dorsally. Once the pupal case started splitting, adult eclosion was completed rapidly (ca. 30 sec). Empty pupal cases were translucent golden brown and retained the frontal horn.

Post-Eclosion Activities

After eclosion, moths ascended vertically (ca. 40 mm or less) to an apical position on a shoot before expanding their wings. Wing expansion began as soon as the adults came to rest and progressed until the wings were completely expanded and slightly elevated above the body. Expansion ended with the quick and complete elevation of the wings to an upright position above the moth's thorax.

From the time eclosion was completed until the wings were fully expanded required an average of 8.7 ± 0.02 min ($\bar{x} \pm t_{.05}\, s_{\bar{x}}$) with a range of 4-16 min measured for 44 moths

(13♂, 31♀). While the wings were in the elevated position, 5.9 ± 0.21 min with a range of 2-12.5 min (13♂, 31♀) were required for drying the wings before they were lowered to rest over the abdomen of the moth. The total time from eclosion, through wing inflation, drying, and until the wings were lowered to rest required an average of 14.6 ± 0.04 min with a range of 7.0-25.0 min (13♂, 31♀).

After the moth's wings were expanded and returned to rest, moths remained motionless in emergence cups until disturbed.

On shoots affixed to the emergence board, the moths periodically moved to a more shaded portion of the shoots as the sun traversed. Charts from the emergence recorder corroborated that only slight periodic movements occurred between the completion of wing expansion and evening flight.

Daily Emergence Patterns

The results of emergence in relation to time of day are given in Table 6 and Figure 11. All critical values of all z test statistics for the Rayleigh Test of randomness (Batschelet 1965) were greater than the tabulated z values at the 0.01 level (Table 6). Therefore, the distribution of the time-of-day emergence data (Figure 11) did not have a uniform distribution, but rather a unimodal or bimodal pattern.

Assuming *R. subtropica* emergence occurred once daily, the mean time of emergence (ϑ) was 0925 EDST with an angular deviation (s) about the mean of ±3.26 hr for non-sexed data,

Table 6

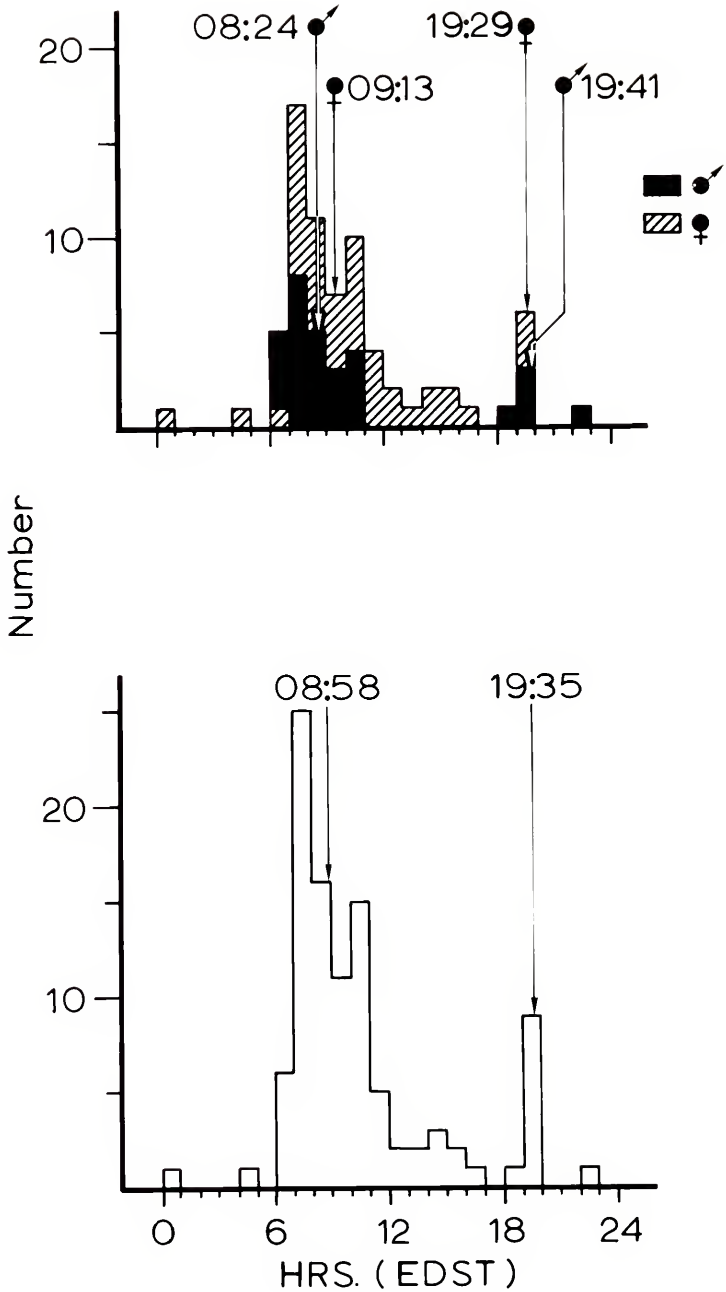
Test Statistics of the Rayleigh Test of Randomness and Calculations of the Mean Emergence Times for Both the Unimodal and Bimodal Emergence of *R. subtropica*

	UNIMODAL EMERGENCE			BIMODAL EMERGENCE					
				MAJOR EMERGENCE			MINOR EMERGENCE		
	♂ + ♀	♂	♀	♂ + ♀	♂	♀	♂ + ♀	♂	♀
r	0.64	0.61	0.64	0.83	0.93	0.78	0.97	0.93	0.99
R	64.96	18.28	42.50	70.77	23.27	47.86	10.63	4.66	5.99
z	41.38	11.13	27.37	58.93	21.66	38.19	10.29	4.34	5.98
n	101[a]	30	66	85	25	60	11	5	6
ϑ	0925	0827	0927	0858	0824	0913	1935	1941	1929
s	±3.26	±3.37	±3.22	±2.21	±1.42	±2.42	±0.81	±1.40	±0.24
Sunrise									
September 2					0708				
September 26					0717				
Sunset									
September 2								1950	
September 26								1919	

[a]5 non-sexed moths included.

Note: r = mean vector; R = test statistic of Rayleigh's test, z = test statistic of randomness; n = number; ϑ = mean time of emergence in hours and minutes; s = angular deviation about the mean in hours and hundredths of hours.

Figure 11. Frequency distributions of *R. subtropica* emergence by hours (EDST = Eastern Daylight Saving Time) of non-sexed moths (n = 101) (bottom) and sexed moths (n = 96). Mean emergence times are indicated above histogram.


08:24
09:13
19:29
19:41
20
10
Number
08:58
19:35
20
10
0
6
12
18
24
HRS. (EDST)

0827 EDST ±3.37 hr for males, and 0927 EDST ±3.22 for females (Table 6). Peak emergence occurred between 0700 and 0800 EDST (Figure 11). The assumption of and the statistics calculated for a unimodal pattern of emergence were not mathematically incorrect, but biologically misleading. The test statistic r, which measures the concentration about the mean and which was not close to the value 1.0, also indicated the emergence was not unimodal.

R. subtropica emerged twice daily and the values of the test statistic r were closer to the value 1.0 for the bimodal calculations. The major emergence occurred just after sunrise with the mean time of emergence of the non-sexed data at 0858 EDST ±2.21 hr, males 0824 EDST ±1.42 hr, and females 0913 EDST ±2.42 hr (Table 6 and Figure 11). The minor emergence was during the evening crepuscular period with the mean time of emergence for non-sexed moths at 1935 EDST ±0.81 hr, males 1941 EDST ±1.40 hr, and females 1929 EDST ±0.24 hr (Table 6 and Figure 11).

Discussion

Although the pre-eclosion movements of *R. subtropica* pupae were only observed in emergence cups and not inside a shoot, similar movement and activities of *R. buoliana* pupae inside a shoot served to elevate the pupa from the base of the pupal chamber, up the silk-lined chamber's walls, up to and out the pre-cut exit hole (Pointing 1961, 1963).

R. subtropica adults had no apparent difficulty escaping from the pupal cases of pupae removed from shoots and held in the emergence cups. *R. buoliana* adults experienced difficulty escaping from pupal cases of pupae removed from buds (Pointing 1961).

The bimodal daily field emergence pattern of *R. subtropica* was strongly similar to the field emergence of *R. buoliana* (Pointing 1961, Green 1965) and peak emergence for both species occurred between 0700 and 0800 EDST.

The early morning eclosion of adults, vulnerability of adults to attacks during the flightless period of wing expansion, and inactivity of adults on the shaded portion of terminals might be exploited by releasing predators which are active in the early morning or by applying chemical adulticides on the shaded portion of terminals for the suppression of *R. subtropica*.

Summary

The activities of *R. subtropica* encased pharate adults prior to eclosion, teneral adults during eclosion, and fully dried adults after eclosion are described. Teneral adults walked a short distance (ca. 40 mm) after eclosion and before expanding and drying their wings. The average time required for wing expansion and drying was 14 minutes. The daily pattern of emergence under natural light cycle was bimodal with peaks at 0858 and 1935 hours EDST.

CHAPTER X

EXPERIMENTAL MATING, OVIPOSITION, AND REARING MEDIA TRIALS OF *RHYACIONIA SUBTROPICA*

Background

In 1970, Daterman described procedures he found useful for mating *Rhyacionia* spp. in the laboratory. In early 1971, correspondence and consultations with G. E. Daterman and C. W. Fatzinger were initiated to obtain the most current information about rearing media for forest insects in general and specifically *R. buoliana* (Daterman, personal communication) and *D. abietella* (Fatzinger 1970a, 1970b).

The objectives of this study were to (1) breed *R. subtropica* moths under artificial or semi-artificial conditions, (2) rear *R. subtropica* larvae on an artificial medium, and (3) establish a laboratory colony of *R. subtropica* larvae and adults.

Methods

Mating and Oviposition

Three mating and oviposition techniques were tested.

Daterman's mating technique. A replica of Daterman's (1970) mating and oviposition apparatus was constructed. The apparatus was designed to simulate natural twilight and to

create an airflow necessary for carrying the female sex pheromone to the male. The light intensities and airflow of the apparatus were calibrated and *R. subtropica* adults were prepared for mating per Daterman's specifications. The mating chamber was located in a rearing room having a north exposure to light, 70% relative humidity, and temperature of 27 ± 2°C. The mating chamber's twilight and photoperiod were synchronized with the natural twilight and photoperiod (Gainesville, Florida). A battery-operated light, dimmed with a red acetate film, was used when entering the rearing room during scotophase.

The initial mating trial was conducted on 16-18 May 1972 using 2 males and 3 females which had emerged in the laboratory from pupae collected in Dixie County, Florida, on 9 May 1972.

Slash pine shoots suspected of bearing *R. subtropica* eggs were incubated in the rearing room on moistened No. 2 Whatman filter paper in a petri dish to determine if oviposition had occurred and if 1st stage larvae would eclose. This mating trial was terminated 29 May 1972.

During September 1972, the mating-oviposition chamber was re-synchronized with the natural photoperiod and operated. Adults for these mating trials were obtained from the previously discussed adult emergence and wing expansion studies. These mating trials were terminated in October 1972.

Fatzinger's mating technique. On 14 July 1972 *R. subtropica* pupae collected in Levy County, Florida, on 11 July 1972 were supplied to C. W. Fatzinger for mating trials

employing his (1970a) *D. abietella* mating and oviposition techniques. Fatzinger terminated the mating trials when all moths were dead (C. W. Fatzinger, personal communication).

Caged-seedling mating technique. From June 1972 to January 1973, wooden-framed screen (100 mesh) cages (.91 x .60 x .51 m), each containing a one-year-old potted slash pine seedling, and maintained in the previously described laboratory rearing room and in a greenhouse (air-conditioned or heated to maintain ca. 27°C) were continuously stocked with *R. subtropica* adults which had recently emerged from pupae periodically collected from throughout Florida. The caged mating trials were terminated during January 1973.

Rearing Media

Two rearing media were tested.

Bedard's modified rearing medium. In April 1972, the pine phloem-based medium of Bedard (1966) was prepared as modified by Richeson et al. (1971) and was evaluated for rearing *R. subtropica* larvae. Non-surface-sterilized 3rd and 4th stage larvae collected 28 March 1972 in Putnam and St. Johns Counties, Florida, were placed 8 larvae per each of 4 petri dishes containing Bedard's medium. The dishes were incubated at 27 ± 2°C with a high humidity (ca. 85%). Pupae which developed were placed in individual plastic cups lined with a moistened disk of No. 2 Whatman filter paper in the previously described rearing room. This rearing medium experiment was terminated 23 April 1972.

Fatzinger's rearing medium. During May 1973, C. W. Fatzinger provided cups of his (1970a) WGCS artificial diet to this author. All stages of *R. subtropica* test larvae were collected in Glades County, Florida, and without being surface-sterilized, were individually placed in 12 cups containing the WGCS diet on 1 June 1973. The cups were incubated in the previously described rearing room. Pupae which developed were placed in individual plastic cups containing an aseptic agar medium (Batcheler and Emmel 1974) as a moisture source and incubated in the rearing room until moths emerged. This rearing medium experiment was terminated during July 1973.

Results

Mating and Oviposition

All mating and oviposition techniques failed.

Rearing Media

The *R. subtropica* larval feeding trials did yield partial success. Six of the 32 larvae fed on Bedard's modified medium (Richeson et al. 1971) and pupated (5 males, 1 female), and 2 of the males emerged. The remainder of the pupae died from desiccation. The use of Batcheler and Emmel's (1974) aseptic agar medium as a moisture source eliminated pupal desiccation during later studies.

The 12 larvae placed on Fatzinger's (1970a) diet fed and pupated. Ten adults emerged during July 1973, in synchrony with the natural field population from which the larvae were

collected. An ichneumonid, *Temelucha* sp. (Hymenoptera: Ichneumonidae),(determined by R. W. Carlson), emerged from each of the other pupae.

Discussion

The failure to achieve mating of *R. subtropica* was not the first time a tip moth species failed to mate under artificial or semi-artificial conditions. Initial laboratory mating trials with *R. buoliana* failed (Chawla and Harwood 1968). C. W. Berisford (personal communication) also constructed a replica of Daterman's (1970) mating chamber and failed to get *R. frustrana*, *R. rigidana*, and *R. subtropica* to mate. Until reliable and successful laboratory mating-oviposition techniques are developed, an aseptic continuously reared *R. subtropica* laboratory colony cannot be established and maintained.

The WGCS medium of Fatzinger (1970a) was the better medium for the production of *R. subtropica* adults. The successful rearing of *R. subtropica* on Fatzinger's WGCS artificial diet was not surprising. *R. frustrana* and *Pissodes nemorensis* Germar (Coleoptera:Curculionidae) larvae reared on the WGCS artificial diet completed development and produced adults; *P. nemorensis* oviposited and initiated a second generation on the diet (C. W. Fatzinger, personal communication).

Summary

R. subtropica adults did not mate under laboratory or semi-artificial conditions. *R. subtropica* larvae fed and developed on the WGCS artificial diet (Fatzinger 1970a) and produced pupae from which adults issued.

CHAPTER XI

HYMENOPTEROUS PARASITES OF *RHYACIONIA SUBTROPICA*

Background

Yates (1967b) and Harman and Kulman (1973) compiled the most current information relating to the parasites of the genus *Rhyacionia* for the Neartic Region and the world, respectively. The objectives of this study were to collect and to identify some of the hymenopterous parasites attacking *R. subtropica* in south Florida.

Methods

From February 1971 through June 1974, *R. subtropica*-attacked slash pine shoots were periodically collected in Florida. Pupae and parasitized larvae were placed in individual emergence cups containing an aseptic agar medium (Batcheler and Emmel 1974) as a moisture source and held in a rearing room at 27 ± 2°C in order that adult parasites could emerge. When superparasitism occurred, larvae and pupae of the particular parasite were preserved. All instars of the parasites were preserved in 70% ethanol. The parasites were taken to E. E. Grissell, who forwarded the braconid, eulophid, and ichneumonid to P. M. Marsh, B. D. Burks, and R. W.

Carlson, respectively, for determination. Grissell determined the eupelmid and chalcidids. C. W. Berisford returned parasites, *Bracon gemmaecola* (Cush.), which emerged from *R. subtropica* pupae I provided Berisford for his *Rhyacionia* sex pheromone studies. The parasites, along with collection and emergence data, were deposited in the Florida State Collection of Arthropods.

Results

The six species of hymenopterous parasites (Table 7) recovered from *R. subtropica* are the first parasites reported for this tip moth (cf. Yates 1967b, cf. Harman and Kulman 1973). Four of the recovered species were new records for Florida (Muesebeck et al. 1951, Krombein 1958, Krombein and Burks 1967, Yates 1967b, Harman and Kulman 1973). Yates (1967b) and Harman and Kulman (1973) also reported *Hyssopus rhyacioniae* Gah. as being gregarious, but *B. gemmaecola* has not been previously reported as being gregarious or having multiple attacks. These parasites, with the exception of *Arachnophaga ferruginea* Gah., are all known parasites of at least one other *Rhyacionia* species (Yates 1967b, Harman and Kulman 1973).

Discussion

Since the six species of hymenopterous parasites reported occur throughout the ranges of other North American *Rhyacionia*

Table 7

Some Hymenopterous Parasites of *R. subtropica* from Florida

	NUMBER OF ADULTS	NUMBER OF PUPAE	NUMBER OF LARVAE	PUPAL PARASITE	LARVAL PARASITE	SINGLE ATTACK	MULTIPLE ATTACKS	MONTH EMERGED	DE SOTO CO.	DIXIE CO.	GLADES CO.	NEW FLORIDA RECORD
BRACONIDAE												
Bracon gemmaecola (Cush.)	35	1	1		+		+	5,6,9	+		+	+
ICHNEUMONIDAE												
Temelucha new species	1			+		+		5			+	+
EULOPHIDAE												
Hyssopus rhyacioniae Gah.	113	11	4		+		+	2,5-7,9,10			+	+
EUPELMIDAE												
Arachnophaga ferruginea Gah.	1			+		+		10			+	+
CHALCIDIDAE												
Haltichella rhyacioniae Gah.	1			+		+		6		+		+
Sphilochalcis flavopicta (Cress.)	25			+		+		6,8-10		+	+	

species and are variously distributed throughout Florida as parasites of *R. subtropica*, the search for additional useful species of parasites of *R. subtropica* and other *Rhyacionia* species should be increased to include the West Indies and Central America.

Summary

Bracon gemmaecola, *Temelucha* new species, *Hyssopus rhyacioniae*, *Arachnophaga ferruginea*, *Haltichella rhyacioniae*, and *Sphilochalcis flavopicta* were recovered from individual *R. subtropica* larvae and pupae. These six species of hymenopterous parasites are the first parasites reported for *R. subtropica*.

CHAPTER XII

INCIDENCE OF *RHYACIONIA SUBTROPICA* IN FERTILIZER-INSECTICIDE EXPERIMENTAL PLOTS

Background

In 1968, The Cooperative Research in Forest Fertilization (CRIFF) program (a joint effort of forest industry, agricultural chemical companies, and the School of Forestry and Department of Soils at the University of Florida) established replicated experimental fertilizer tests (A-Series, uniform fertilizer experiments on young stands) in the lower coastal plains of the southeastern United States. W. L. Pritchett, CRIFF Coordinator, Department of Soils, University of Florida, has records of the objectives and information relative to the CRIFF program.

At the Austin Cary Forest of the University of Florida, located in Alachua County, Florida, one-half of the experimental plots of 2-year-old bedded slash pine seedlings were fertilized with 90 Kg P/ha in April 1969 (C. M. Kaufman, personal communication).

In the summer of 1969, tip moths, *Rhyacionia* sp., infested >7% of the trees in the CRIFF A-Series potassium test sites (Anonymous 1970). *R. frustrana* infested >90% of all bedded trees in Kaufman's phosphorous and non-phosphorous plots but

almost all non-bedded (control) seedlings were free from infestation (R. C. Wilkinson, personal communication).

These separate but simultaneous tip moth infestations in variously treated slash pine plantings prompted the planning by R. C. Wilkinson (personal communication) and Hudson Pulp and Paper Corporation personnel to establish research plots near Relay, Florida, to study the relationship of insect pests (tip moths in particular) to slash pine maintained under various fertilizer regimes. The Relay Tract plots were established to duplicate current seed orchard fertilizer practices, and certain systemic insecticide treatments which had proven successful for the control of tip moths (Barras et al. 1967).

Objectives of this study were (1) to determine if various fertilizer-insecticide combinations applied to bedded slash pine seedlings would affect the incidence of *R. subtropica*, (2) to determine the distribution of *R. subtropica* larval feeding sites within the multi-nodal annual vegetative long shoot (apical terminals), and (3) to determine the incidence of *R. subtropica* by tree height classes.

Methods

Experimental Plots

Design. In December 1969, P. E. Lavely and R. C. Wilkinson (personal communication) outlined the design for the fertilizer-insecticide experimental plots.

Three fertilizer treatments and a check were replicated 3 times in a randomized block design of 40-tree plots. Each plot (40 trees) was split, and one-half of each plot (20 trees) was treated with the same insecticide as a control for *R. subtropica* (P. D. Kidd, personal communication).

Location. The experimental plots were located in the pollen isolation strip surrounding and adjacent to the northern boundary of a seed orchard located east of the abandoned settlement of Relay, Flagler County, Florida (Section 2, Township 14 South, Range 31 East) (Figure 12) (P. D. Kidd, personal communication). The study area is referred to as the Relay Tract plots.

Establishment. In January 1970, selected 1-0 slash pine nursery stock was hand-planted to insure seedlings were (1) properly planted on beds, (2) free of disease, and (3) not attacked by *Rhyacionia* spp. To prevent damage by cattle, and possible injury to cattle or persons from the insecticide applications, the Relay Tract plots were enclosed by a three-strand barbed wire fence and posted as being an "Experimental Area" (P. D. Kidd, personal communication.

Formulation and application of treatments. The fertilizer and phorate (0,0-diethyl-S-[(ethylthio)=methyl] phosphorodithioate) insecticide treatments were based on the fertilizer recommendations of W. L. Pritchett and the insecticide recommendations of R. C. Wilkinson, and were applied per the formulations and schedule outlined in Table 8. All treatments were applied to the soil at the base of each tree. P. W.

Figure 12. Relay Tract plots. Annotated composite of USCGS Codys Corner and Favoretta, Florida, 7.5 minute series (topographic) quadrangle maps illustrating the location of the seed orchard (Seed Orchard) and fertilizer-insecticide experimental plots (Plots) in Section 2, Township 14 South, Range 31 East, Flagler County, Florida. This location is about 10 miles south of the city of Bunnell in northeastern coastal Florida (see Figure 2).

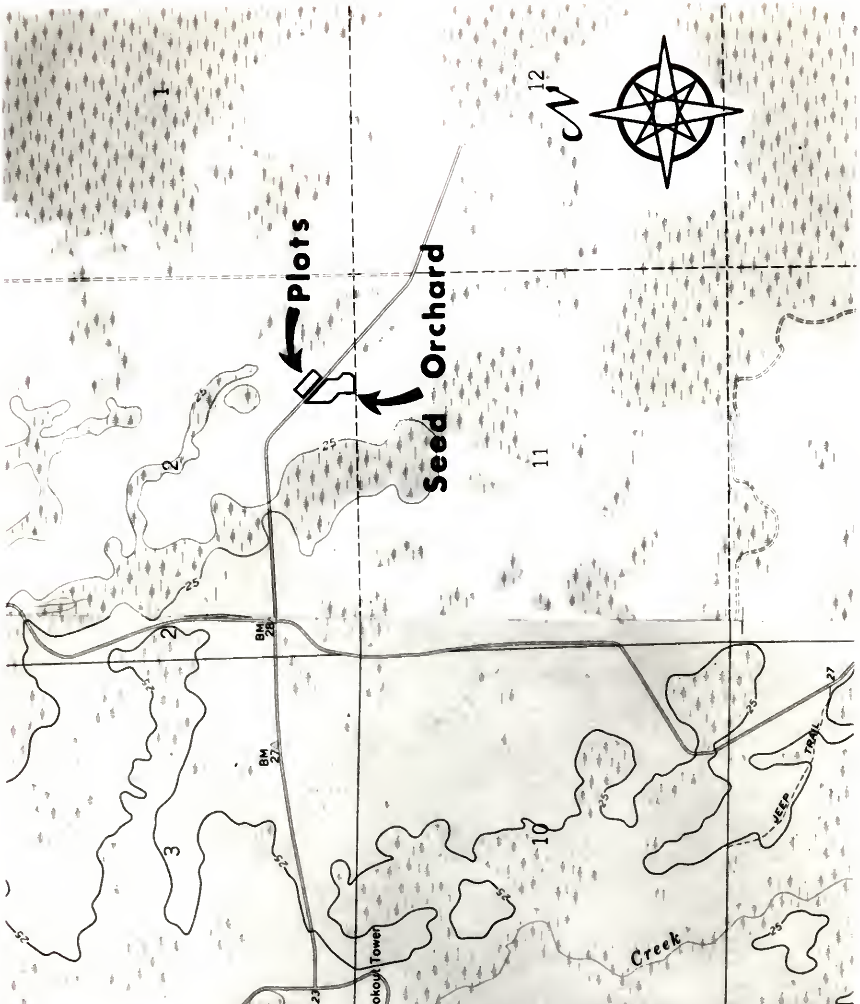

Plots
Seed Orchard
Lookout Tower
Relay
Middle
How
Creek
JEEP
TRAIL
BM 28
BM 27
BM 23
1
2
3
4
9
10
11
12

Table 8

Application Dates and Rates of Fertilizer and Phorate Applied to Soil at the Base of Each Bedded Slash Pine Seedling in the Relay Tract Plots During 1970 and 1971

TREATMENT	FORMULATION	APPLICATION DATE AND RATE	
		JANUARY 1970	MARCH 1971
		(gm/seedling)	
N-O-O	NH_4NO_3	62.4	
	$CO(NH_2)_2$		95.7
O-P-K[a]	N-P_2O_5-K_2O[b]	62.4	
	$Ca(H_2PO_4)_2$		122.3
	KCl		93.6
N-P-K	NH_4NO_3	62.4	
	N-P_2O_5-K_2O[b]	62.4	
	$CO(NH_2)_2$		76.3
	$Ca(H_2PO_4)_2$		122.3
	KCl		93.6
O-O-O	(CHECK)		
PHORATE[c]	10% granular[d]	25	75

[a]Only P and K applied after 1970; hence treatment designation O-P-K.

[b]10-10-10 (N-P-K) commercial fertilizer.

[c]O,O-diethyl-S-[(ethylthio)=methyl]phosphorodithioate.

[d]One-half of each 40-tree plot received phorate per schedule.

Lavely supervised the 1970 installation and application, and E. M. Underhill supervised the 1971 application (P. D. Kidd, personal communication).

Plant parts were not analyzed for either N, P, K, or the insecticide phorate and its analogs to determine the amount of chemical uptake by the slash pine seedlings.

Incidence of *R. subtropica*

General. Between 10 and 17 June 1971, all 1971 growth present (apical and lateral shoots) on each 2-year-old seedling in all 24 Relay Tract plots was examined for the presence of *R. subtropica* larval feeding. After the end of the growing season, the total 1971 growth (apical and lateral shoots) was re-examined from 28-30 December 1971 and any additional *R. subtropica*-infested seedlings were added to the June tally.

Incidence of *R. subtropica* by treatment. Each seedling was tallied as either infested or not infested by *R. subtropica* during 1971. Chi-square analysis of the data was conducted to determine (1) within a particular fertilizer treatment, did the experimental application of phorate affect the percentage of seedlings infested? (2) disregarding the fertilizer treatments, did the experimental application of phorate affect the percentage of seedlings infested? and (3) which of the 8 treatments had the lowest percentage of infested seedlings?

Distribution of larval feeding sites within apical terminals. The data of this study were recorded at the same time as the data for the incidence of infestation data. Larval feeding sites on seedlings with infested apical terminals were recorded according to the flush (1st, 2nd, 3rd, and/or 4th) within the terminal infested and according to the site (bottom, middle, top) infested within each flush.

Incidence of *R. subtropica* by tree height classes. In January 1974, the total 1971 height (feet) of all remaining 1971 attacked and unattacked seedlings was measured. The frequency distribution of *R. subtropica* infestations among seedlings was graphed in one-foot tree height classes (e.g., 1.5-2.4 ft. = 2-ft. height class).

Results

Incidence of *R. subtropica* by Treatment

Results of the χ^2 analyses concerning the percent incidence of 1971 *R. subtropica*-infested seedlings are given in Table 9. These percentages were derived from counts presented in Table 10 to eliminate the effect of small differences in the number of trees surviving in each plot.

In the N-O-O and the N-P-K treatments, the split plots receiving the phorate had a significantly higher percentage of infested seedlings. The percentage of infested seedlings in the O-P-K and O-O-O treatments was not significantly reduced by the application of phorate.

Table 9

Chi-Square Analyses of Percent Incidence of 2-Year-Old Bedded Slash Pine Seedlings Infested by *R. subtropica* in the Relay Tract Plots During 1971

PLOT TREATMENTS	SPLIT-PLOT TREATMENTS		AVERAGE	χ^2	LEVEL OF SIGNIFICANCE
	PHORATE	NO PHORATE			
O-O-O	23.33	30.00	26.67	0.68	0.50
N-O-O	39.66	62.07	50.86	5.82	0.025
O-P-K	22.41	36.67	29.66	2.87	0.10
N-P-K	40.00	60.38	49.56	4.67	0.05
AVERAGE	31.36	46.75		24.16	0.0005
χ^2	7.89	18.63	11.64		
LEVEL OF SIGNIFICANCE	0.05	0.0005	0.001		

Table 10

Numbers of 2-Year-Old Bedded Slash Pine Seedlings Infested (n=182) and Not Infested (n=285) by *R. subtropica* in the Relay Tract Plots During 1971

PLOT TREATMENT	SPLIT-PLOT TREATMENTS			
	PHORATE		NO PHORATE	
	INFESTED	NOT INFESTED	INFESTED	NOT INFESTED
0-0-0	14	46	18	42
N-0-0	23	35	36	22
0-P-K	13	45	22	38
N-P-K	24	36	32	21
TOTAL	74	162	108	123

When the four fertilizer treatments were disregarded, plots which received the phorate had a significantly lower percentage of infested seedlings.

The 0-P-K and the 0-0-0 treatments had the lowest percentages of infested seedlings and the percentage of infested seedlings within each fertilizer treatment was not significantly reduced by the application of phorate. The 0-P-K treatment with phorate had the lowest, but not significantly lowest, percentage of infested seedlings.

No apparent phytotoxic effects were noted from the experimental use of phorate in this experiment.

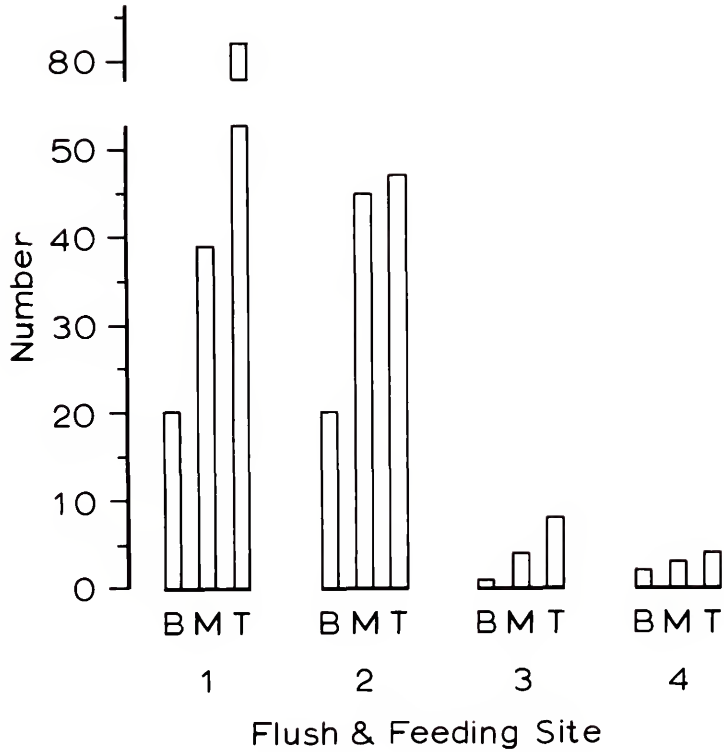
Distribution of Larval Feeding Sites Within Apical Terminals

Of the 182 seedlings infested by *R. subtropica*, 160 had larval feeding sites on their apical terminals. The total number of feeding sites, recorded by point of attack (bottom, middle, top) within each flush was 275 (Figure 13).

The periods of slash pine 1st, 2nd, and 3rd flush elongation recorded in north Florida during 1971 were mid-March (ca. 19th), early May (ca. 5th), and variously throughout June, respectively. The periods of *R. subtropica* 1st and 2nd generation emergence recorded in north Florida during 1971 were mid-February (ca. 16th) and variously throughout June, respectively.

The 1st (141) and 2nd (112) flushes received 92% of the *R. subtropica* attacks. The 3rd and 4th flushes accounted for 4.7 and 3.3% of the total *R. subtropica* feeding sites,

Figure 13. Frequency distribution, by site (b = bottom; m = middle; t = top) and without regard to treatments, of the number of *R. subtropica* larval feeding sites (n = 275) on the 1st four flushes of the apical terminals of 2-year-old bedded slash pine seedlings (n = 160) in the Relay Tract plots during 1971.


Number
80
50
40
30
20
10
0
B M T
B M T
B M T
B M T
1
2
3
4
Flush & Feeding Site

respectively. The apical terminals of 27 seedlings were attacked twice, and 24 of the double attacks were on the 1st and 2nd flushes.

Within a flush, the upper portions (middle and top) of each flush had the majority of the larval feeding sites.

Incidence of *R. subtropica* by Tree Height Classes

The frequency distributions of the 1971 *R. subtropica*-infested seedlings and the total number of seedlings is graphed by tree height classes in Figure 14.

Of the 467 seedlings present in 1971, only 453 were present in January 1974 when the 1971 total height was measured. Six 1971 *R. subtropica*-infested and 8 non-infested seedlings had died in the interim.

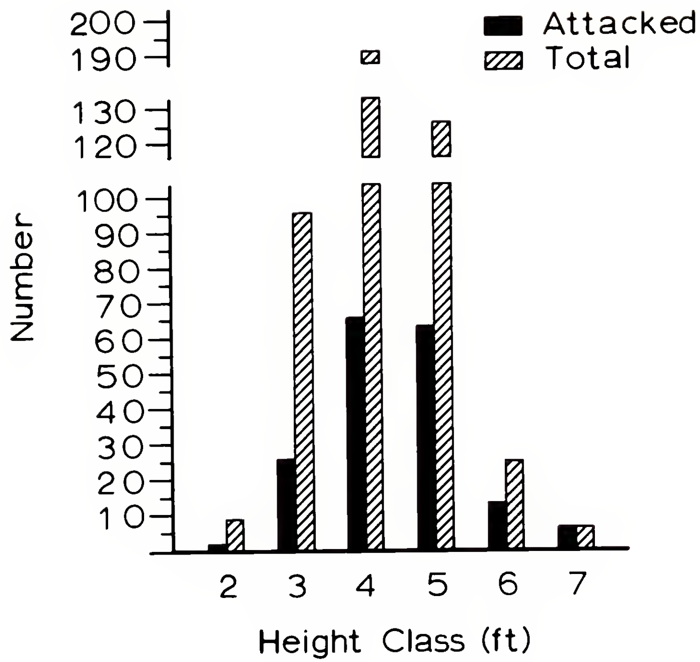
Both the total number of seedlings and the number of 1971 *R. subtropica*-infested seedlings have apparently binomial tree height class distributions, and the two distributions occur in superimposition.

As total height increased, a greater percentage of the seedlings within each height class (22.2, 27.1, 34.3, 51.2, 52.0, and 100.0% for height classes 2 through 7, respectively) were infested by *R. subtropica* during 1971.

Discussion

In 1971, *R. subtropica* infested a higher percentage of the seedlings receiving N (N-O-O, N-P-K) than those seedlings not receiving N (O-P-K, O-O-O). The application of N

Figure 14. Frequency distributions, by height class and without regard to treatments, of the number of *R. subtropica*-infested (n = 176) and total number (n = 453) of 2-year-old bedded slash pine seedlings in the Relay Tract plots during 1971.


Attacked
Total
Number
200
190
130
120
100
90
80
70
60
50
40
30
20
10
2
3
4
5
6
7
Height Class (ft)

apparently "predisposed" the seedlings to infestation by *R. subtropica*. The application of phorate did offer some protection to those seedlings receiving N. The incidence of *R. buoliana* in scotch pine (*P. sylvestris* L.) in Europe was also increased by applications of N (NO_3, NH_4, N, NP) fertilizers and reduced by application of a N-P-K-Mg-Ca fertilizer (Schindler 1967).

The O-P-K treatments had statistically lower percentages of *R. subtropica*-infested seedlings than the N treatments, but not a statistically lower percentage of infested seedlings than the control (0-0-0). The application of P and K (0-P-K) did not "protect" seedlings better than no fertilizer (0-0-0), but the 0-P-K treatment did have the lowest percentage of infestations within the plots receiving some type of fertilizer. Therefore, from the standpoint of the three fertilizer treatments, the 0-P-K treatment had the lowest percentage of *R. subtropica*-infested seedlings.

The low percentage of seedlings infested by *R. subtropica* in the experimental phorate plots was comparable to the results obtained by Barras et al. (1967) and Yates (1970) when they experimentally applied similar amounts of phorate to control *R. frustrana* and *Rhyacionia* spp., respectively, infesting loblolly pines.

The disproportionately large number of *R. subtropica* attacks on the 1st and 2nd flushes or the decline in number of attacks on the 3rd and 4th flushes of the slash pine seedlings during the 1971 study could have occurred because

(1) the 1st flush was subject to all 1st generation *R. subtropica* larval feeding; (2) during June, when the 2nd generation *R. subtropica* oviposition occurred, a majority of the seedlings had an elongated 2nd flush, only a few seedlings had an elongating 3rd flush, and no seedlings had a 4th flush; and (3) a natural decline comparable to what Yates (1966) reported in his study when *Rhyacionia* spp. injury to slash pine shoots declined during June and became difficult to find after the last of July.

Hughes and Jackson (1962) reported *D. amatella* infested a greater percentage of the slash pine seedlings within a height class as total height increased, similar to the increased infestation per height class recorded for *R. subtropica* during 1971.

Summary

The 0-P-K and the 0-0-0 treatments, either with or without phorate, were superior to any of the N-0-0 or N-P-K treatments for the avoidance of infestation by *R. subtropica* to bedded 2-year-old slash pine seedlings during 1971. When the four fertilizer treatments were disregarded, plots which received phorate had a significantly lower percentage of *R. subtropica*-infested seedlings.

R. subtropica larval feeding sites were recorded more frequently in the upper portion of a flush and most frequently on the first two flushes of the apical terminal.

The percentage of *R. subtropica*-infested slash pine seedlings within a height class increased as total 1971 height increased.

CHAPTER XIII

RHYACIONIA SUBTROPICA AND PITCH CANKER IN SLASH PINE

Background

Pitch canker of pines (Hepting and Roth 1946) is caused by *Fusarium lateritium* f. *pini* (Nees.) Hepting (Snyder et al. 1949). Hepting and Roth (1946) described pitch canker symptoms as external copious pitch flow and internal pitch soaked wood, and Laird and Chellman (1972) presented color photographs of these symptoms.

The pathogenicity of the fungus (Anonymous 1957, Artman 1973, Hepting 1961,1971, Hepting and Roth 1953), geographic distribution (Hepting and Roth 1946,1953, Berry and Hepting 1959,1969, Snyder et al. 1949), role of the fungus in stimulating oleoresin flow (Anonymous 1951, Clapper 1954, Hepting 1947,1954, True and Snow 1949), spread of the disease within pine stands (Anonymous 1956,1958, Bethune and Hepting 1963, Laird and Chellman 1972, Schmidt and Underhill 1974), and the taxonomic status of the fungus (Booth 1971) have been reported. The infrequent presence of fungal fruiting bodies on pitch cankers in nature (Berry and Hepting 1959,1969, Hepting and Roth 1946, Snyder et al. 1949, Stegall 1966), the repeated occurrence of initial symptoms on the current year's apical

shoots (Bethune and Hepting 1963), the association of infections with insect feeding scars or injury (Berry and Hepting 1959, Hepting and Roth 1953, Snyder et al. 1949), and the random occurrence of newly diseased trees within pine stands (Schmidt and Underhill 1974) have led to the thesis that insects are involved in the pitch canker disease cycle (Berry and Hepting 1959,1969, Hepting 1971, Hepting and Roth 1953, Schmidt and Underhill 1974). Matthews (1962) specifically investigated the association of pitch canker and *Rhyacionia* spp. on slash pine in Florida.

After pitch canker outbreaks in slash pine plantations (Laird and Chellman 1972, Schmidt and Underhill 1974) and in a slash pine seed production area (Schmidt and Underhill 1974) in Florida during 1969, R. C. Wilkinson (personal communication), R. A. Schmidt, and Hudson Pulp and Paper Corporation personnel jointly initiated research to investigate the relationship of forest insect pests and pitch canker in slash pines in Florida.

The objective of this investigation was to examine the association of *R. subtropica* and pitch canker in slash pines in Florida.

Studies were conducted to determine (1) the association of *R. subtropica* attacks and pitch canker symptoms, (2) the distribution of *R. subtropica* larval feeding sites and pitch canker symptoms on annual multinodal vegetative long shoots (apical terminals), (3) the incidence of pitch canker in bedded slash pines maintained under various fertilizer-

insecticide treatments designed to duplicate current seed orchard fertilizer practices and certain systemic insectitide treatments which were successful for the control of *Rhyacionia* spp. (Barras et al. 1967), (4) the association of pitch canker-producing *Fusarium* spp. and *R. subtropica* on pairs of slash pine shoots, and (5) the incidence of pitch canker-producing *Fusarium* spp. isolated from *R. subtropica* instars.

Methods

Relay Tract Field Studies

General. In December 1969, P. E. Lavely and R. C. Wilkinson (personal communication) outlined the design for the fertilizer-insecticide experimental plots.

Three fertilizer treatments and a check were replicated 3 times in a randomized block design of 40-tree plots. Each plot was split, and one-half of each plot (20 trees) was treated with the same insecticide as a control for *R. subtropica* (P. D. Kidd, personal communications).

The experimental plots, referred to as Relay Tract plots, were located in the pollen isolation strip surrounding and adjacent to the northern boundary of a pitch canker-infested seed orchard located east of the abandoned settlement of Relay, Flagler County, Florida (Section 2, Township 14 South, Range 31 East) (Figure 12) (P. D. Kidd, personal communication). The pitch canker study plots of Schmidt and Underhill (1974) were located in the adjacent slash pine plantations.

In January 1970, selected 1-0 slash pine nursery stock was hand-planted to insure seedlings were (1) properly planted on beds, (2) free of disease, and (3) not attacked by *Rhyacionia* spp. To prevent damage by cattle, and possible injury to cattle or persons from the insecticide applications, the Relay Tract plots were enclosed by a 3-strand barbed wire fence and posted as being an "Experimental Area" (P. D. Kidd, personal communication).

The fertilizer and phorate (O,O-diethyl-S-[(ethylthio)= methyl] phosphorodithioate) insecticide treatments were based on the fertilizer recommendations of W. L. Pritchett and the insecticide recommendations of R. C. Wilkinson, and were applied per the formulations and schedule outlined in Table 11. All treatments were applied to the soil at the base of each tree. P. W. Lavely supervised the 1970 installation and application, and E. M. Underhill supervised the remainder of the applications from 1971-1974 (P. D. Kidd, personal communication).

Plant parts were not analyzed for either N, P, K, or the insecticide phorate and its analogs to determine the amount of chemical uptake by the slash pine seedlings.

Association of *R. subtropica* attacks and pitch canker symptoms. In June and December 1971, all 1971 apical shoots on each pine in the 24 plots were examined and the exact location of all *R. subtropica* injury and damage was recorded. In May 1973, the loci of 1971 *R. subtropica* injury and damage were re-examined for the presence of pitch canker symptoms. In April 1974, all 1973 shoots (lateral and apical) which

Table 11

Application Dates and Rates of Fertilizer and Phorate Applied to Soil at the Base of Each Bedded Slash Pine in the Relay Tract Plots From 1970 Until 1974

TREATMENT	FORMULATION	DATE & RATE (gm/tree) OF APPLICATION				
		JAN 1970	MAR 1971	MAR 1972	APR 1973	MAY 1974
N-O-O	NH_4NO_3	62.4				
	$CO(NH_2)_2$		95.7	138.7	138.7	138.7
O-P-K[a]	N-P_2O_5-K_2O[b]	62.4				
	$Ca(H_2PO_4)_2$		122.3	135.9	135.9	135.9
	KCl		93.6	103.9	103.9	103.9
N-P-K	NH_4NO_3	62.4				
	N-P_2O_5-K_2O[b]	62.4				
	$CO(NH_2)_2$		76.3	138.7	138.7	138.7
	$Ca(H_2PO_4)_2$		122.3	135.9	135.9	135.9
	KCl		93.6	103.9	103.9	103.9
O-O-O	(check)	0	0	0	0	0
Phorate[c]	10% granular[d]	25	75	75	75	75

[a]Only P and K applied after 1970; hence treatment designation O-P-K.

[b]10-10-10 (N-P-K) commercial fertilizer.

[c]O,O-diethyl-S-[(ethylthio)=methyl]phosphorodithioate.

[d]One half of each plot received phorate per schedule.

developed pitch canker symptoms since May 1973 were examined for signs of *R. subtropica* damage.

Distribution of *R. subtropica* larval feeding sites and pitch canker symptoms on apical shoots of slash pines. In December 1971, the distribution of *R. subtropica* larval feeding sites on apically-infested 1971 shoots was recorded by internode (1st, 2nd, 3rd, and/or 4th) within the shoot. In April 1974, the distribution of external pitch canker symptoms on apically-diseased 1973 shoots was recorded according to the node (1st, 2nd, 3rd, or 4th) within the shoot. No pitch canker shoots from the trees within the Relay Tract plots were destructively examined for comparison of the locations of external and internal symptoms, but pitch canker shoots from trees outside the Relay Tract plots were so examined. The frequency distributions of the 1971 internodes infested by *R. subtropica* and the 1973 nodes with pitch canker symptoms were constructed.

Incidence of pitch canker symptoms in relationship to fertilizer and insecticide treatments. In April 1974, the 1973 growth on each pine was marked as either diseased or not diseased with pitch canker. Chi-square analysis of the data was conducted to determine (1) within a particular fertilizer treatment, did the experimental application of phorate affect the percentage of diseased trees? (2) disregarding the fertilizer treatments, did the experimental application of phorate affect the percentage of diseased trees? and (3) which of the 8 treatments had the lowest percentage of diseased trees?

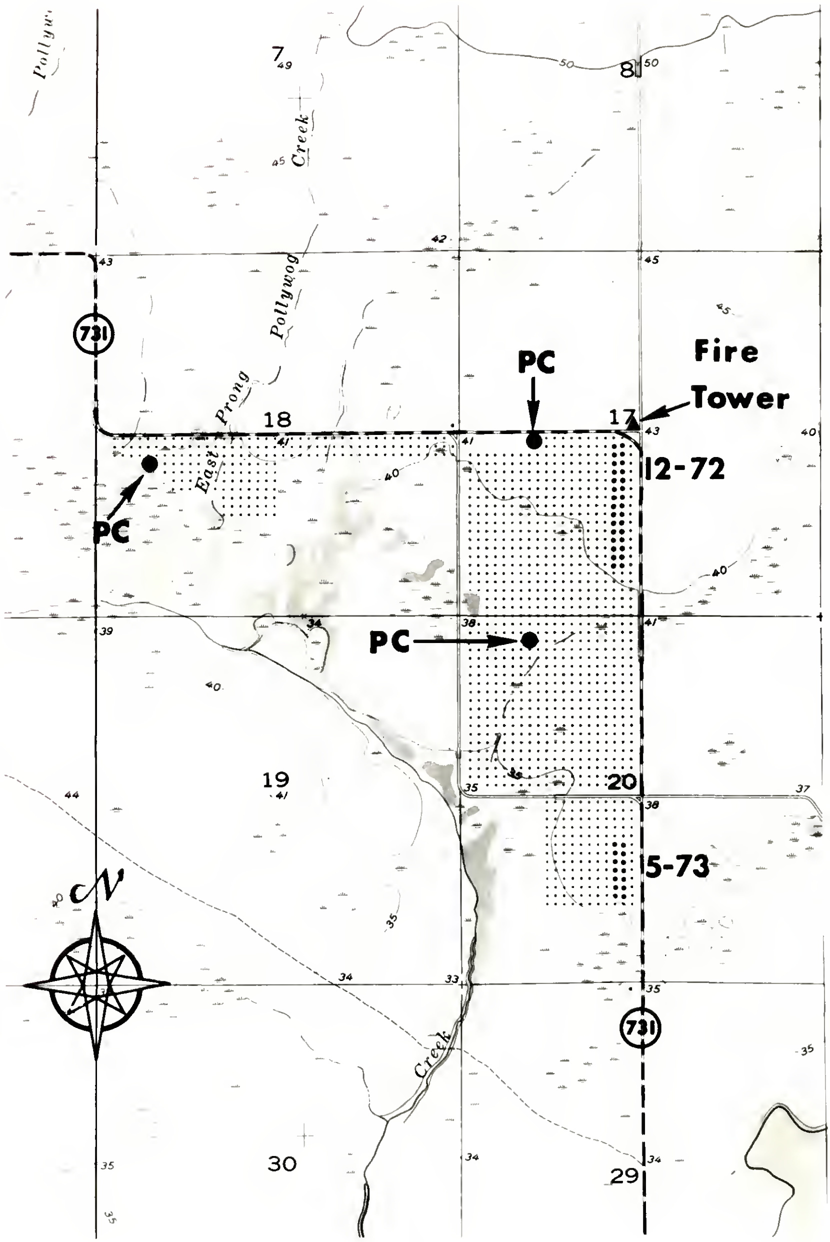
Glades County Field Study

Association of *R. subtropica* attacks and pitch canker symptoms. In December 1972, 300 1972 *R. subtropica*-damaged slash pine shoots (lateral and apical) were flagged on 2-year-old bedded pines located in Section 17, Township 42 South, Range 29 East in southwestern Glades County, Florida. In May 1973, 200 1973 *R. subtropica*-injured slash pine shoots (lateral and apical) were flagged on pines located in Section 20 of the same plantation (Figure 15). The locations of pitch canker infections on residual south Florida slash pines within and adjacent to the plantation containing the flagged pines were mapped. In December 1973, all 500 flagged shoots were re-examined for the presence of pitch canker disease symptoms.

Laboratory Studies

Association of pitch canker-producing *Fusarium* spp. and *R. subtropica* on pairs of slash pine shoots. From each of 20 disease-free young slash pines, a *R. subtropica*-infested apical shoot and an adjacent non-*R. subtropica*-infested shoot, each ca. 20 cm long, were collected in Glades County, first during July 1971, and again during May 1973. Needles were stripped from each shoot without touching the portion to be collected, a coded, sterile plastic bag was placed over the defoliated shoot, a 70% ethanol-swabbed knife was used to remove the shoot, and the plastic bag containing the shoot was sealed and placed on ice for return to the laboratory.

Figure 15. Glades County plots. Annotated USCGS La Belle, Florida, 7.5 minute series (topographic) quadrangle map illustrating the location of the December 1972 and May 1973 *R. subtropica*-attacked slash pine plots (larger dots) in Sections 17 and 20, respectively. The smaller dots represent extent and boundaries of the slash pine plantation. The locations of pitch canker infections (PC), Sections 17, 18, and 20, on residual south Florida slash pines within or adjacent to the slash pine plantation are noted. The Lykes lookout tower (Fire Tower) is located in the center of Section 17, Township 42 South, Range 29 East, about 4 miles north of the city of La Belle in southern Florida (see Figure 2).


Pollywog
7
49
8
50
Creek
45
42
43
45
731
Pollywog
Fire
Tower
PC
Prong
18
17
43
40
41
41
East
40
12-72
PC
40
39
34
38
41
PC
40
19
41
44
35
20
37
38
5-73
N
35
34
33
35
731
Creek
35
35
30
34
29
34
35

The shoots were incubated in the plastic bags in a room at 27 ± 2°C with a 12-hr light:dark photoperiod. The plastic bags were opened under aseptic conditions and aerial mycelium was removed from the exterior of the shoots and transferred to Difco potato-dextrose agar medium (PDA) for further incubation. *Fusarium* spp. isolates were identified with the aid of a compound microscope (magnification 400X). Single spores of *Fusarium* spp. were transferred to PDA slants by the procedure of Toussoun and Nelson (1968). Mycelium was removed from the single-spore pure cultures by aseptic techniques, transferred to long grain white rice in sterilized test tubes, and incubated at 27 ± 2°C.

Each rice-cultured *Fusarium* spp. isolate was inoculated into 5 succulent, disease-free slash pine shoots under aseptic conditions. After removing the shoot's foliage at the point of inoculation and swabbing the stem with 70% ethanol, colonized rice was placed in a "T" slit, and the slit was closed, covered with cotton, and wrapped with tape. In addition, 5 shoots were inoculated with a *Fusarium* spp. isolate which had previously produced pitch canker symptoms and 5 shoots were inoculated with non-inoculated rice.

Inoculated shoots were allowed to incubate for a maximum of 6 weeks or until pitch canker symptoms appeared. The wrappings were removed and the shoots were examined for external and internal pitch canker symptoms.

Chi-square tests of independence (Snedecor and Cochran 1967) were conducted to determine if a difference existed in

the incidence of *Fusarium* spp. and/or *Fusarium* spp. which produced pitch canker from *R. subtropica*-infested and non-*R. subtropica*-infested shoots.

Incidence of pitch canker-producing *Fusarium* spp. isolated from *R. subtropica* instars. *R. subtropica*-attacked slash pine shoots were collected in Glades County in south Florida during September 1973. In the laboratory, *R. subtropica* larvae and pupae were removed from infested shoots with sterilized forceps and placed inside sterilized petri dishes. The design of this study is illustrated in Figure 16.

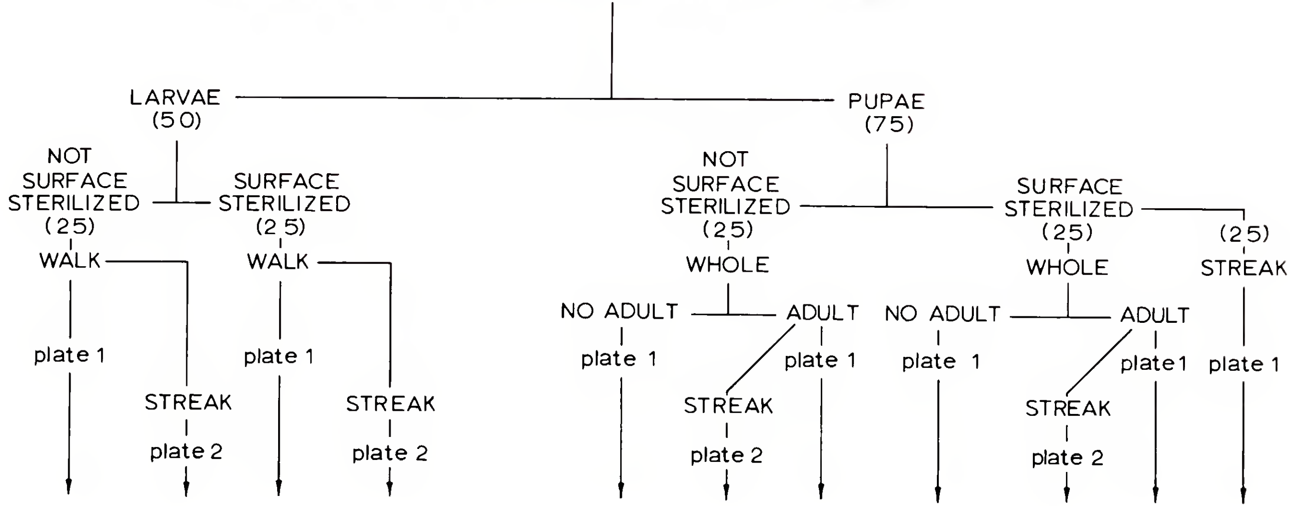
Living larvae, 25 unsterilized and 25 surface sterilized in modified White's solution (Barras 1972), were placed in separate petri dishes containing peptone-pentachloronitrobenzene (PCNB) agar (Nash and Snyder 1962). After 12 hr, the living larvae were removed and individually streaked onto peptone-PCNB agar of a second petri dish.

Living pupae, 25 unsterilized and 25 surface-sterilized in modified White's solution, were placed in separate petri dishes containing peptone-PCNB agar. Adults which emerged were removed and individually streaked onto peptone-PCNB agar of a second petri dish. In addition, 25 living pupae were surface sterilized in modified White's solution and individually streaked onto peptone-PCNB agar in petri dishes. All dishes were incubated at 27 ± 2°C with 12-hr light:dark photoperiod.

The purification, production of inoculum, inoculation of slash pine shoots, and examination for pitch canker symptoms

Figure 16. Diagram of procedure employed to obtain *Fusarium* spp. isolates from *R. subtropica* larvae and pupae removed from slash pine shoots collected in Glades County, Florida.

Rhyacionia subtropica instars from ***Pinus elliottii*** var. ***elliottii***



were conducted as previously described, except 3 shoots instead of 5 shoots were inoculated.

Results

Field Studies

Association of *R. subtropica* attacks and pitch canker symptoms. In the Glades County plots, neither the 300 1972 *R. subtropica*-damaged slash pine shoots (Section 17) nor the 200 1973 *R. subtropica*-injured slash pine shoots (Section 20) developed pitch canker symptoms (Table 12).

In the Relay Tract plots, none of the 1971 sites of *R. subtropica*-injured or damaged shoots on the 182 attacked trees developed pitch canker symptoms, and none of the 70 1973 shoots (lateral or apical) of the 70 trees which developed pitch canker symptoms between 16 May 1973 and 26 April 1974 had *R. subtropica* damage (Table 13).

Distribution of *R. subtropica* larval feeding sites and pitch canker symptoms on apical shoots of slash pines. The frequency distributions of *R. subtropica* larval feeding sites on the internodes of 1971 apical shoots and pitch canker symptoms at nodes of 1973 apical shoots of slash pines in the Relay Tract plots are shown in Figure 17.

During 1971, 160 of the 182 slash pines infested by *R. subtropica* had 275 larval feeding sites on their apical shoots. The frequency of larval feeding was 141, 112, 13, and 9 for the 1st, 2nd, 3rd, and 4th internodes, respectively.

Table 12

The Number of 1972 and 1973 *R. subtropica*-Attacked Slash Pine Shoots in the Glades County Plots Which Developed Pitch Canker During 1973

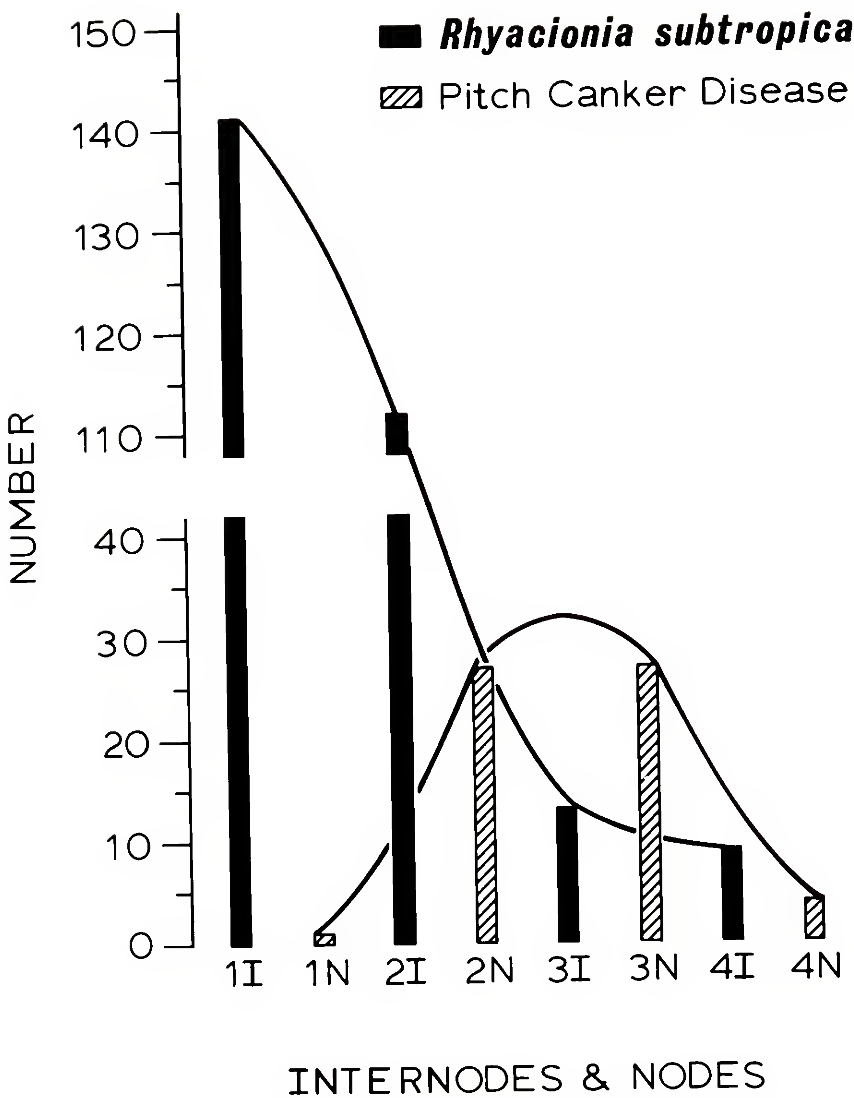
	1972	1973	TOTAL
	--(number of shoots)--		
R. SUBTROPICA-ATTACKED	300	200	500
PITCH CANKER SYMPTOMS	0	0	0

Table 13

Number of 1971 *R. subtropica*-Attacked Shoots Which Developed Pitch Canker and the Number of 1973 Pitch Canker-Infested Shoots Which Had *R. subtropica* Damage

	1971	1973-74
	--(number of shoots)--	
EXAMINED	467	441
R. SUBTROPICA-ATTACKED	182	0
PITCH CANKER SYMPTOMS	0	70

Figure 17. Frequency distribution of 275 *R. subtropica* larval feeding sites on the internodes (I) of 1971 apical shoots and 70 pitch canker symptoms at the nodes (N) of 1973 apical shoots of slash pines in the Relay Tract plots.


Rhyacionia subtropica
Pitch Canker Disease
NUMBER
150
140
130
120
110
40
30
20
10
0
1I
1N
2I
2N
3I
3N
4I
4N
INTERNODES & NODES

During 1973-74, 59 of the 70 slash pines which developed pitch canker had symptoms on their 1973 apical shoots. In contrast to the internodal occurrence of *R. subtropica* attacks, these symptoms were located at nodes along the apical shoots (Figure 18) and the frequency of occurrence was 1, 27, 27, and 4 for the 1st, 2nd, 3rd, and 4th nodes, respectively.

The majority of the 1971 *R. subtropica* larval feeding sites (253) occurred on the 1st and 2nd internodes; in contrast the majority of the 1973-74 pitch canker symptoms (58) occurred at the 2nd, 3rd, and 4th nodes. The sites of *R. subtropica* larval feeding and pitch canker symptoms did not occur at similar locations on apical slash pine shoots either in time or space.

Incidence of pitch canker in relationship to fertilizer and insecticide treatments. Results of the χ^2 analyses of the percent of trees with pitch canker in relation to fertilizer and insecticide treatments during 1973-74 in the Relay Tract plots are given in Table 14. Trees receiving phorate had a significantly lower percentage of pitch canker than trees not receiving phorate. Within the 4 fertilizer treatments, the 0-P-K and 0-0-0 phorate split plots had significantly less pitch canker than the non-phorate split plots. The percentage of pitch canker in the N-0-0 and N-P-K treatments was unaffected by the application of phorate.

With regard to all 8 fertilizer-insecticide treatments, the highest percentages of diseased trees were in the 0-P-K and N-P-K non-phorate treatments, and the lowest percentages

Figure 18. De-barked apical slash pine shoots showing pitch canker symptoms (pitch-soaked wood) occurring at the branch nodes.

Table 14

Chi-Square Analyses of the Percent Incidence of Pitch Canker in Fertilizer-Insecticide Treated 4-Year-Old Slash Pines in the Relay Tract Plots During 1973-74

FERTILIZER TREATMENTS	INSECTICIDE TREATMENTS		AVERAGE	χ^2	LEVEL OF SIGNIFICANCE
	PHORATE	NO PHORATE			
	(percent)				
O-O-O	0.00	17.54	8.62	11.33	0.001
N-O-O	3.85	13.24	8.57	2.94	0.10
O-P-K	5.26	30.00	18.10	11.86	0.001
N-P-K	22.81	36.17	28.85	2.24	0.20
AVERAGE	8.04	23.96		20.94	0.0005
χ^2	23.76	9.70	22.30		
LEVEL OF SIGNIFICANCE	0.0005	0.025	0.0005		

of diseased trees were in the N-0-0, 0-P-K, and 0-0-0 phorate treatments. The trees in the 0-0-0 phorate treatment had no pitch canker.

In general, increased fertility and the absence of phorate were associated with the increased incidence of pitch canker.

Laboratory Studies

Association of *Fusarium* spp., *Fusarium* spp. which produced pitch canker symptoms, and *R. subtropica* on pairs of slash pine shoots. The incidence of *Fusarium* spp. isolated from *R. subtropica*-attacked and non-attacked paired shoots from 40 slash pines in Glades County, Florida, during 1971 and 1973, and subsequent pitch canker symptoms produced by inoculation in healthy slash pines are shown in Table 15.

During 1971, no *Fusarium* spp. isolates were obtained from the 20 disease-free shoots not attacked by *R. subtropica*, but *Fusarium* spp. isolates were obtained from 15 of the 20 disease-free shoots attacked by *R. subtropica*. The χ^2 test of independence at the 1% level of significance with χ^2 = 24.00 confirmed *Fusarium* spp. were highly associated with *R. subtropica*-attacked slash pine shoots. Healthy slash pine shoots were not inoculated with the *Fusarium* spp. isolates to determine if those isolates would produce pitch canker symptoms.

During 1973, no *Fusarium* spp. isolates were obtained from the 20 disease-free shoots not attacked by *R. subtropica*,

Table 15

The Incidence of *Fusarium* spp. Isolated from *R. subtropica*-Attacked and Non-attacked Paired Shoots from 40 Slash Pines in Glades County, Florida, During 1971 and 1973 and Subsequent Pitch Canker Symptoms Produced by Inoculation in Healthy Slash Pines

	RHYACIONIA SUBTROPICA ATTACKED[a]			
	1971		1973	
	ABSENT	PRESENT	ABSENT	PRESENT
	(number of shoots)			
INCUBATED	20	20	20	20
YIELDING *FUSARIUM* SPP.	0	15	0	14
YIELDING *FUSARIUM* SPP. WHICH PRODUCED PITCH CANKER SYMPTOMS	--	--	0	2

[a]Symptoms of pitch canker absent at time of collection.

confirming the results of the 1971 study. *Fusarium* spp. isolates were obtained from 14 of 20 disease-free shoots attacked by *R. subtropica* and the χ^2 test of independence at the 1% level of significance with $\chi^2 = 21.56$ again confirmed *Fusarium* spp. were highly associated with *R. subtropica*-attacked slash pine shoots. Two of the 14 *Fusarium* spp. isolates produced pitch canker symptoms when healthy slash pine shoots were inoculated. The χ^2 value for the test of independence of *R. subtropica* and *Fusarium* spp. isolates which produced pitch canker symptoms was 2.05. A χ^2 value greater than 2.05 is expected to occur about 15% of the time; therefore, the association of *R. subtropica* and *Fusarium* spp. isolates which produced pitch canker symptoms cannot be accepted or rejected.

Incidence of pitch canker-producing *Fusarium* spp. isolated from *R. subtropica* instars. The incidence of *Fusarium* spp. isolated from *R. subtropica* larvae and pupae removed from slash pine shoots collected in Glades County, Florida, and subsequent pitch canker symptoms produced by those isolates are shown in Table 16.

A total of 16 of the 25 non-surface-sterilized *R. subtropica* larvae, 11 larvae in the walk treatment, and 10 larvae in the streak treatment, yielded *Fusarium* spp. isolates. Seven of 11 walk and 4 of 10 streak *Fusarium* spp. isolates produced pitch canker symptoms upon inoculation of healthy slash pine shoots.

Table 16

The Incidence of *Fusarium* spp. Isolated from *R. subtropica* Instars Removed from Slash Pine Shoots Collected in Glades County, Florida, and Subsequent Pitch Canker Symptoms Produced by Those Isolates

	RHYACIONIA SUBTROPICA INSTARS						
	LARVAE				PUPAE		
	NON-SURFACE STERILIZED		SURFACE STERILIZED		NON-SURFACE STERILIZED	SURFACE STERILIZED	
	WALK	STREAK	WALK	STREAK	WHOLE	WHOLE	STREAK
	(number)						
INCUBATED	25	25	25	25	25	25	25
YIELDING *FUSARIUM* SPP.	11	10	6	4	15	1	0
YIELDING *FUSARIUM* SPP. WHICH PRODUCED PITCH CANKER SYMPTOMS	7	4	4	2	12	1	0

A total of 6 of the 25 surface-sterilized *R. subtropica* larvae, 6 larvae in the walk treatment, and 4 larvae in the streak treatment,yielded *Fusarium* spp. isolates. Four of 6 walk and 2 of 4 streak *Fusarium* spp. isolates produced pitch canker symptoms upon inoculation of healthy slash pine shoots.

Fifteen of the 25 non-surface-sterilized *R. subtropica* pupae yielded *Fusarium* spp. isolates and 12 of those *Fusarium* spp. isolates produced pitch canker symptoms upon inoculation of healthy slash pine shoots.

One of the 25 surface-sterilized, whole *R. subtropica* pupae yielded a *Fusarium* spp. isolate and that isolate produced pitch canker symptoms upon inoculation of healthy slash pine shoots. None of the 25 surface-sterilized, streaked *R. subtropica* pupae yielded *Fusarium* spp. isolates.

Fusarium spp. isolates were associated with *R. subtropica* larvae and pupae, and most frequently with non-surface-sterilized instars. *Fusarium* spp. isolates which produced pitch canker symptoms upon inoculation of healthy slash pine shoots were more frequently obtained from the exterior of (walk or whole) larvae and (whole) pupae and never from the interior of (streak) surface-sterilized pupae. Neither adults which issued from whole, surface-sterilized pupae nor streaked, surface-sterilized pupae yielded *Fusarium* spp. isolates which produced pitch canker symptoms upon inoculation of healthy slash pine shoots.

Discussion

Based on the results of the field and laboratory investigations of the relationship between *R. subtropica* and pitch canker, the following conclusions are discussed with respect to the minimal requirements of Leach's (1940) "Rules of Proof for Insect Transmission [of plant pathogens]," p. 541.

Rule 1 states: "A close, although not necessarily a constant, association of the insect with diseased plants must be demonstrated." Field studies demonstrated (1) *R. subtropica*-attacked slash pine shoots did not develop pitch canker, (2) slash pine shoots with pitch canker symptoms did not have signs of *R. subtropica*, and (3) the distributions of *R. subtropica* larval feeding sites and pitch canker symptoms did not occur at similar locations on slash pine apical shoots either in time or space. Laboratory isolations and inoculations demonstrated *R. subtropica* injury on slash pine was associated with *Fusarium* spp. and *Fusarium* spp. which produced pitch canker symptoms ca. 70 and 10% of the time, respectively. Therefore, neither a constant nor a close association of *R. subtropica* with pitch-cankered slash pines was demonstrated.

Rule 2 states: "It must be demonstrated that the insect also regularly visits healthy plants under conditions suitable for the transmission of the disease." The conditions, environmental and physiological, suitable for the oviposition (visitation) of *R. subtropica* female moths on or transmission

of *F. lateritium* f. *pini* to slash pine shoots are not known nor were the conditions investigated. Pitch canker infections (Bethune and Hepting 1963, Stegall 1966) and *R. subtropica* infestations (see Chapter XII) are both known to occur on the living tissues of a current year's vegetative long shoot (apical or lateral terminal) of pine. Although the presence of *R. subtropica* larvae in host plant tissues suitable for colonization by *F. lateritium* f.*pini* was demonstrated, the absence of pitch canker symptoms on those *R. subtropica*-attacked slash pine shoots demonstrated that *R. subtropica* female moths had not visited the slash pine shoots under conditions suitable for the transmission of *F. lateritium* f. *pini*.

Rule 3 states: "The presence of the pathogen or virus in or on the insect in nature or following visitation to a diseased plant must be demonstrated." Laboratory isolations and inoculations verified that pitch canker-producing *Fusarium* spp. were associated with the exterior of *R. subtropica* larvae and pupae removed from within the microenvironment of slash pine shoots. Pitch canker-producing *Fusarium* spp. were obtained neither from the interior of surface-sterilized pupae, which would eventually become adults, nor from adults which issued from surface-sterilized pupae. Although the presence of pitch canker-producing *Fusarium* spp. on *R. subtropica* larvae and pupae in nature was demonstrated, the presence of pitch canker-producing *Fusarium* spp. on or in potential

or actual morphological forms, adults, which are capable of moving between potential hosts was not demonstrated.

Rule 4 states: "The disease must be produced experimentally by insect visitation under controlled conditions with adequate checks." The absence, during laboratory studies, of pitch canker-producing *Fusarium* spp. on or in potential adults of *R. subtropica*, the absence of functional mouth parts in the adult stage of *R. subtropica*, and mating and oviposition as the sole functions performed by *R. subtropica* females in nature suggest that *R. subtropica* adults are not capable of frequently transmitting *F. lateritium* f. *pini* or successfully inoculating slash pine shoots with *F. lateritium* f. *pini* in nature. When artificial laboratory techniques are developed for the successful mating and oviposition of *R. subtropica*, the visitation and oviposition of female *R. subtropica* moths artificially contaminated with *F. lateritium* f. *pini* could experimentally demonstrate under controlled laboratory conditions if the transmission of *F. lateritium* f. *pini* could occur.

The results of these studies differ in part with previously reported results concerning a possible relationship between *Rhyacionia* spp. and pitch canker. The field studies demonstrated an absence of an association between *R. subtropica* and pitch canker contrary to previously reported statements (Bethune and Hepting 1963, Berry and Hepting 1969). The laboratory studies showed that *Fusarium* spp. were highly associated with both *R. subtropica*-attacked slash pine shoots and non-surface-sterilized *R. subtropica* larvae and pupae

removed from slash pine shoots; but inoculations of healthy slash pines, which Mathews (1962) apparently did not perform, failed to demonstrate that the majority of those *R. subtropica*-associated *Fusarium* spp. could produce pitch canker symptoms.

Since the field and laboratory studies of the relationship between *R. subtropica* and pitch canker did not demonstrate *R. subtropica* fulfilled the minimal requirements for the transmission of the pitch canker pathogen, *R. subtropica* is concluded not to be a prime agent in the initiation and spread of pitch canker in slash pine in Florida.

However, during the field studies, a sequence of observed events and one particular documented result did strengthen the thesis that some insect(s) is probably involved in the pitch canker disease cycle (Hepting and Roth 1953, Berry and Hepting 1969, Schmidt and Underhill 1974).

First, the initial incidence of pitch canker in the Relay Tract area during 1969 was abrupt and apparently randomly distributed within the pine plantations (Schmidt and Underhill 1974). From January 1970 [when the Relay Tract plots were established] until May 1973 [when 1971 loci of *R. subtropica* injury and damage were last examined for the presence of pitch canker symptoms], the pines in the Relay Tract plots were free of pitch canker. But, between May 1973 and December 1973, pitch canker invaded the Relay Tract plots abruptly and was randomly distributed within the individual plots, with one exception. Additional pitch canker symptoms continued to periodically occur on 1973 shoots until after

the 1974 apical shoots had initiated in March 1974. This 2-1/2 years of absence of pitch canker, the rapid and random re-occurrence of pitch canker, and the infrequent presence of pitch canker fruiting bodies in nature (Berry and Hepting 1959,1969, Hepting and Roth 1946, Snyder et al. 1949, Stegall 1966) suggest that a vector could be involved in the pitch canker disease cycle.

Secondly, the incidence of pitch canker was significantly lower in all phorate-treated plots and pitch canker was totally absent in the non-fertilized, phorate-treated plots. These facts suggest two theses: (1) phorate possessed fungicidal properties which were toxic to the pitch canker fungus, or (2) phorate reduced the incidence of an insect(s) which is involved in the disease cycle of the pitch canker fungus.

The fact that slash pines which received phorate had significantly less pitch canker provides sufficient circumstantial evidence to (1) suggest that some insect(s)[1] (other than *R. subtropica*) is involved in the initiation and spread of pitch canker in slash pine in Florida, and (2) justify additional pitch canker-vector studies.

Summary

Field studies demonstrated (1) *R. subtropica*-attacked slash pine shoots did not develop pitch canker, (2) slash pine shoots with pitch canker symptoms did not have signs of

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[1]Observations (J. R. McGraw) and preliminary studies (R. C. Wilkinson, personal communication) suggest that thrips which inhabit slash pine shoots could be associated with pitch canker in slash pine in Florida.

R. subtropica attacks, (3) the distributions of *R. subtropica* larval feeding sites and pitch canker symptoms did not occur at similar locations on slash pine apical shoots, and (4) slash pines which received applications of phorate had less pitch canker than slash pine which did not receive applications of phorate.

Laboratory studies demonstrated pitch canker-producing *Fusarium* spp. were (1) associated infrequently with *R. subtropica*-infested slash pine shoots, (2) associated with *R. subtropica* non-surface-sterilized larvae, surface-sterilized larvae, and non-surface-sterilized pupae, and (3) not associated with either the interior of surface-sterilized pupae or adults which issued from surface-sterilized pupae.

Thus, *R. subtropica* is not considered as a prime agent in the initiation and spread of pitch canker in slash pines in Florida.

CHAPTER XIV

SUMMARY AND CONCLUSIONS

A biological study of *R. subtropica*, a tip moth which attacks young pines in the southeastern United States, the West Indies, and Central America, was undertaken. This study was conducted mainly in Flagler County in northeastern Florida and Glades County in southwestern Florida from January 1971 until July 1974.

R. subtropica was collected attacking pines in southern Georgia, on Grand Bahamas Island, on Sugarloaf Key (the most southern site pines grow in the United States), and throughout Florida; and was most abundant in the southern and coastal areas of Florida.

R. subtropica has 5 larval instars and the 4th and 5th female larval instars have paired secondary sex structures on their 8th and 9th abdominal sternites. These secondary sex structures were used to demonstrate 5th larval instar female head capsules were larger than the head capsules of 5th larval instar males.

In south Florida, *R. subtropica* was bivoltine with a partial 3rd emergence in September and October, and the seasonal histories of both *R. subtropica* and slash pine exhibited intersynchronization.

First and 2nd stage *R. subtropica* larvae fed on foliar tissues or tissues associated with foliar tissues. The 3rd, 4th, and 5th stage *R. subtropica* larvae tunneled into the internal tissues of vegetative long shoots and apical buds. Injury was generally associated with 1st and 2nd stage larval feeding, and damage was caused by 3rd, 4th, and 5th stage larval feeding. *R. subtropica* did not usually produce deformities in slash pines grown in north Florida, but usually caused the deformity of slash pines growing in south Florida.

R. subtropica larval feeding sites were recorded more frequently in the upper portion of a flush and most frequently on the first two flushes of the apical shoots. The percentage of infested slash pine seedlings within a height class increased as total height of 2-year-old seedlings increased.

The mature 5th stage *R. subtropica* larvae constructed silk-lined pupal chambers in and prepared pupal exit holes along the apical portions of mined shoots. In multiple-infested shoots, pupation generally proceeded downwardly with an apparently random distribution of pupal sex. The weight by sex, sex ratio, colors, and duration of pupae are also reported.

The activities of encased pharate *R. subtropica* adults prior to eclosion, teneral adults during eclosion, and fully dried adults after eclosion are described. Teneral adults walked a short distance (ca. 40 mm) after eclosion and before expanding and drying their wings. The average time required for wing expansion and drying was ca. 14 min. The daily

pattern of emergence under natural light cycle was bimodal, with major and minor peaks at 0858 and 1935 hours EDST, respectively.

R. subtropica adults did not mate under laboratory or semi-artificial conditions, but field-collected larvae fed and developed on WGCS artificial diet and produced pupae which issued adults.

Six species of hymenopterous parasites, *Bracon gemmaecola*, *Temelucha* new species, *Hyssopus rhyacioniae*, *Arachnophaga ferruginea*, *Haltichella rhyacioniae*, and *Sphilochalcis flavopicta*, were recovered from individual *R. subtropica* larvae and pupae. These parasites are the first parasites reported for *R. subtropica*.

On bedded 2-year-old slash pines maintained with 8 combinations of fertilizer and insecticide treatments, the O-P-K and O-O-O treatments, either with or without phorate, were superior to any of the N-O-O or N-P-K treatments for the avoidance of infestation by *R. subtropica*. Seedlings which received phorate had a significantly lower percentage of infested seedlings.

The association of *R. subtropica* and pitch canker was also investigated. Field studies demonstrated *R. subtropica* attacked slash pine shoots did not develop pitch canker, slash pine shoots with pitch canker symptoms did not have signs of *R. subtropica* attacks, the distribution of *R. subtropica* larval feeding sites and pitch canker symptoms did not occur at similar locations on slash pine apical shoots

and, in general, increased fertility and the absence of phorate were associated with an increased incidence of pitch canker. Laboratory studies demonstrated pitch canker-producing *Fusarium* spp. were associated infrequently with *R. subtropica*-infested slash pine shoots; associated with *R. subtropica* non-surface-sterilized larvae, surface-sterilized larvae, and non-surface-sterilized pupae; and not associated with either the interior of surface-sterilized pupae or adults which issued from surface-sterilized pupae.

A common name, subtropical pine tip moth, for *R. subtropica* has been submitted to the Committee on Common Names of Insects of the Entomological Society of America.

The following conclusions are drawn as a result of these studies:

R. subtropica probably occurs throughout southeastern coastal North America, the West Indies, and Central America wherever pines grow.

R. subtropica is usually bivoltine in southeastern coastal North America.

Some parasites and predators of other North American *Rhyacionia* species also attack *R. subtropica*.

Within the natural range of slash pine, neither fertilized nor unfertilized commercial slash pines are infested or damaged by *R. subtropica* to an extent to warrant the use of insecticide solely for the control of *R. subtropica*.

Within the natural range of south Florida slash pine, slash pines are repeatedly infested and damaged by

R. subtropica to an extent to warrant not establishing commercial slash pine plantations or seed orchards in those areas.

R. subtropica is not considered as the prime agent in the initiation and spread of pitch canker in slash pines in Florida.

Future studies should include location of *R. subtropica* oviposition sites and determination of the egg incubation period; collection and identification of additional parasites and predators of *R. subtropica*; determination of morphological characters for the taxonomic separation of *R. subtropica* and *R. rigidana* larvae; investigation of possible secondary sex structures on larvae of other *Rhyacionia* and lepidopterous species; investigation of calling time(s) and mating behavior of *R. subtropica*; development of *R. subtropica* laboratory mating techniques; evaluation of the importance of *R. subtropica* in slash pine seed orchards; evaluation of pine-shoot infesting insects, particularly thrips, as possible agents involved in the initiation and spread of pitch canker in slash pine in Florida; and more comprehensive investigations of the effect of insects and diseases in fertilized commercial slash pine plantations.

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BIOGRAPHICAL SKETCH

James Robert McGraw was born September 24, 1943, at Winston-Salem, North Carolina. He attended elementary school in Forsyth County and was graduated from Richard J. Reynolds High School in 1961.

He attended North Carolina State University, Raleigh, North Carolina, from September 1961 until August 1968, where he was awarded degrees of Bachelor of Science in Forest Management and Master of Science in Entomology. He was also selected for membership in Xi Sigma Pi, Phi Sigma Society, and the Society of the Sigma Xi honoraries.

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On August 16, 1969, he married the former Janet Wilson Rawlings of Winston-Salem, North Carolina.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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March, 1975

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